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GLUTATHIONE CONTENT OF BLOOD IN CHRONIC ARTHRITIS AND RHEUMATOID CONDITIONS

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THE encouraging therapeutic results obtained with parenterally given colloidal sulphur in cases of chronic nonspecific arthritis and rheumatoid conditions[1] prompted this investigation as a first step in the study of sulphur metabolism in these diseased states. Since the discovery of glutathione by Hopkins in 1920, much work has been centered around this interesting sulphur compound which represents a thermostabile oxidation-reduction system, and which is assumed to have some important part in the physiologic oxidation-reduction processes of the cells. Normal human blood contains from 30 to 40 mg. of glutathione per 100 c.c.; it seemed of interest to investigate whether or not possible derangements in the sulphur metabolism of our patients are expressed in abnormal glutathione values. By the method described below, the amounts of glutathione were determined in the blood of 28 healthy individuals, of 27 patients with hypertrophic arthritis, of 18 with atrophic arthritis, and of 8 with bursitis and periarthritis (fibrositis). At the close of this experimental work it was noted that the Arthritis Committee of the British Medical Association in a recent report recommended this subject, among others, for research in the study of arthritis.[2]

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ANALYTICAL METHOD*

For the determination of reduced glutathione in blood, Tunnicliffe introduced the iodometric titration of blood filtrates obtained by deproteinization with trichloracetic acid.[3] Numerous modifications of this method were evolved, mainly because iodine oxidizes under prevailing conditions not only glutathione but also ergothioneine, another substance with a sulphhydril group always present in blood. Woodward and Fry[4] made the valuable observation that at high degrees of acidity and low temperature only a small fraction of ergothioneine is affected by iodine. In this laboratory it was found that the presence of a considerable excess of potassium iodide during titration further depresses the iodine consumption of ergothioneine, so that error from this source becomes negligible. The assertion of Woodward and Fry, that in the Folin-Wu method of deproteinization reduced glutathione is rapidly oxidized due to the slight acidity of the filtrate, was not substantiated. No change was found when the Folin-Wu filtrates were allowed to stand for as long as three hours; even after standing all night at room temperature the decrease in reduced glutathione was but slightly over 20 per cent. This observation agrees with that of Benedict and Gottschall[5] who worked with

TABLE I

REDUCED GLUTATHIONE IN THE BLOOD OF HEALTHY INDIVIDUALS

NAME	SEX	AGE	CELL VOLUME PER CENT	GLUTATHIONE IN 100 C.C. OF	
				WHOLE BLOOD	CORPUSCLES
B. K.	F	26	46	39	84
B. W.	F	35	38	33	86
I. D.	F	26	40	39	97
L. M.	F	39	39	30	78
M. B.	F	22	41	38	94
K. M.	F	25	46	40	88
M. S.	F	22	43	46	106
M. B.	F	33	37	41	110
A. F.	F	30	42	49	118
E. K.	F	33	39	39	100
N. J.	F	28	43	43	100
A. B.	F	23	43	38	89
C. S.	F	21	37	40	107
E. S.	M	21	45	37	82
J. T.	M	25	44	45	101
S. S.	M	24	48	37	77
A. S.	M	24	46	47	101
B. C.	M	22	45	37	84
B. D.	M	25	48	41	86
A. S.	M	43	46	39	85
C. S.	F	30	37	31	85
B. P.	M	24	50	41	83
L. K.	M	20	52	36	69
A. S.	F	13	40	34	85
B. F.	F	36	36	36	100
M. H.	M	64	40	45	111
A. N.	M	51	44	31	71
L. N.	F	35	35	29	82
Average			42.6	36.5	91.3

*This method was devised by Dr. Michael Somogyi under whose guidance the analytical work was carried out.

blood filtrates of about the same acidity as that of the Folin-Wu filtrates. In the iodometric titration the excellent Okuda technic[6] was adopted.

The analytical procedure, accordingly, has the following course: Protein-free blood filtrate is prepared by a slightly modified Folin-Wu method[7] in 1:10 dilution; separate the precipitate without undue delay by centrifugation, pour the protein-free fluid through a dry filter paper. Pipette 20 c.c. of the filtrate (which corresponds to 2 c.c. of blood) into an Erlenmeyer flask (100-150 c.c.) and add 1 c.c. of N/1 sulphuric acid. (At this stage the sample may be allowed to stand until a convenient time for titration.) Before titration, introduce 0.3 gm. of potassium iodide and 3 drops of 0.5 per cent starch solution (Lintner's soluble starch), cool the mixture in ice water and from a microburette titrate with 0.0025 N potassium iodate, or biniodate, until the faintest perceptible blue color becomes permanent.

$$\frac{\text{c.c. iodate used} \times 307}{8} = \text{mg. reduced glutathione per 100 c.c. of blood.}$$

All the reagents employed in this analysis keep indefinitely. One-tenth normal potassium biniodate may be prepared as stock solution and 1:40 dilutions of it made from time to time. The potassium iodide may be added in the form of 3 c.c. of a 10 per cent solution but it must not be older than one day.

The results of our experiments are presented in Tables I to V.

TABLE II

REDUCED GLUTATHIONE IN HYPERTROPHIC ARTHRITIS

NAME	SEX	AGE	CELL VOLUME PER CENT	GLUTATHIONE IN 100 C.C. OF	
				WHOLE BLOOD	CORPUSCLES
A. S.	M	52	47	46	98
P. T.	F	48	35	39	111
B. M.	M	60	45	44	98
R. G.	F	58	36	36	98
S. W.	F	52	38	41	108
C. P.	F	39	36	33	92
Y. E.	F	54	40	40	101
E. S.	F	50	44	41	93
S. B.	F	42	43	37	85
R. S.	F	57	43	41	96
B. W.	F	41	36	25	69
A. S.	F	44	45	38	87
W. F.	M	60	49	38	78
P. K.	M	51	44	41	94
E. L.	F	53	42	42	100
J. W.	M	77	44	42	96
R. C.	F	38	37	36	99
B. S.	F	49	38	34	88
L. H.	F	56	38	40	106
B. C.	F	42	42	40	96
E. C.	F	46	42	27	65
A. Z.	F	57	41	33	80
E. W.	F	68	40	42	104
D. Y.	F	68	43	40	93
J. B.	F	51	40	31	79
M. S.	M	60	47	40	86
L. P.	F	45	31	39	123
Average			41	38.1	93.4

In Table I are recorded the glutathione determinations in the blood of 28 healthy individuals of both sexes and of varying ages, for comparison with the results obtained on patients which, grouped according to the class of disease, are given in Tables II to V.

TABLE III

REDUCED GLUTATHIONE IN ATROPHIC ARTHRITIS

NAME	SEX	AGE	CELL VOLUME PER CENT	GLUTATHIONE IN 100 C.C. OF	
				WHOLE BLOOD	CORPUSCLES
L. L.	F	38	32	36	114
M. H.	F	34	40	39	96
B. B.	F	32	37	40	107
M. W.	F	47	37	36	97
S. B.	M	31	37	36	96
M. F.	M	53	30	26	86
M. W.	F	43	42	35	84
S. H.	F	45	39	39	100
J. A.	F	50	37	40	107
R. B.	F	46	35	35	100
N. P.	F	29	37	35	93
S. B.	F	45	36	31	86
D. B.	F	45	40	37	93
R. U.	F	55	40	32	81
R. S.	F	19	44	32	74
R. K.	F	20	37	37	98
R. S.	F	33	43	38	88
G. M.	F	29	40	50	124
Average			37.9	36.2	95.7

TABLE IV

REDUCED GLUTATHIONE IN FIBROSITIS

NAME	SEX	AGE	CELL VOLUME PER CENT	GLUTATHIONE IN 100 C.C. OF	
				WHOLE BLOOD	CORPUSCLES
M. G.	F	50	40	35	88
R. G.	F	58	38	35	92
M. L.	F	31	41	40	97
D. C.	M	42	46	46	100
A. S.	F	43	38	37	98
M. M.	M	58	43	38	89
A. U.	F	61	36	41	114
A. P.	M	52	51	46	89
Average			41.7	39.9	95.9

TABLE V

COMPARISON OF VARIATIONS AND AVERAGES OF THE REDUCED BLOOD GLUTATHIONE IN NORMAL INDIVIDUALS AND IN CASES OF CHRONIC ARTHRITIS AND FIBROSITIS

TYPE OF CASES	NUMBER OF CASES	CELL VOLUME PER CENT	AVERAGE	MG. PER CENT WHOLE BLOOD	AVERAGE	MG. IN 100 C.C. OF CORPUSCLES	AVERAGE
Normal individuals	28	35.2 to 52.6	42.6	28.8 to 49.1	36.5	69 to 118	91.3
Hypertrophic arthritis	27	31.5 to 49.0	41.0	24.9 to 46.1	38.1	69 to 123	93.4
Atrophic arthritis	18	30.0 to 43.8	37.9	25.7 to 49.7	36.2	74 to 124.3	95.7
Fibrositis	8	36.0 to 51.4	41.7	34.9 to 46.3	39.9	88 to 114	95.9

Since the entire glutathione content of blood is confined exclusively within the corpuscles, we have expressed the values both for 100 c.c. of whole blood and for 100 c.c. of corpuscles. Influenced by the same consideration, Gabbe[8] calculated the relation between glutathione and red cell count, while Woodward and Fry attempted to correlate the glutathione and the hemoglobin content. In my opinion, however, none of these forms of expression contributed added information beyond the figures given for whole blood.

The reduced glutathione content of the blood of healthy individuals, calculated for whole blood varied in these experiments between 29 and 49 mg. per 100 c.c., with an average of 36.5 mg. Woodward and Fry[4] obtained with their own method 34, Benedict and Gottschall[5] with a colorimetric procedure found 35 mg. as the average values which are in close agreement with ours.

CONCLUSION

The tables disclose, without need for comment, that the glutathione content in the blood of 27 patients with hypertrophic arthritis, of 18 with atrophic arthritis, and of 8 with fibrositis, compared with that in the blood of 28 normal individuals, shows no appreciable deviations either in regard to the range of variations or in the average values.

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PNEUMOCOCCUS CHAIN FORMATION*

Its Relation to Virulence in Mice, Occurrence and Distribution by Pneumococcus Types in Human Respiratory Infections, and Its Relation to Prognosis in Lobar Pneumonia

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LONG chains of pneumococci in the broth inoculated with the organisms producing the septicemia of mice, resulting from inoculations with pneumonic sputum is a striking phenomenon; its occurrence and significance were studied by us during the period from 1929 to 1933. It seemed to us that there is some correlation between the severity of the disease in patients and the finding of growth of this character in cultures of pneumococci recovered from sputum, lung juice and blood cultures. The microscopic observation of all cultures is imposed by the method of typing employed in our laboratory.

Organisms in the stained smear of the sputum cannot, without further tests, be conclusively classified as pneumococci even though they resemble pneumococci. The determination of their solubility in bile necessitates waiting for the culture.

Chain formation, or chaining of pneumococci, has been observed since 1893 when B. Issaef[1] and J. Washbourn[2] found that pneumococci formed chains when they were grown in homologous specific immune sera. Similar observations were made on *B. pyocyaneus* by Charrin and Roger (1889)[3] and on *B. coli* and *B. proteus* by Pfaundler.[4] When *B. coli* and *B. proteus* grew chainwise the organisms were club-formed, convoluted and stained unevenly, some failing to take the stain. The usual turbidity was present in cultures grown in broth with normal serum, but in the presence of specific immune sera, there was a heavy turbidity in the bottom of the tube and the broth was clear. The normal morphology and cultural characteristics of the organisms were restored by reseeding them in the usual broth.

A study of the relation of chain formation to virulence of the pneumococci in mice was undertaken. Pneumococcus Type IV (Cooper) cultures recovered from Case 4,613 were passed through mice daily for eight days. One cubic centimeter of the eighteen-hour culture from the mouse heart's blood was used. With each succeeding passage the chains became shorter; on the eighth day there were only occasional short chains of from three to five diplococci. The rest were in pairs. The first mouse lived twenty hours and the eighth mouse only seven hours.

A Type I culture isolated from Case 4,717 was passed through mice nine days before the chains completely disappeared. The first mouse lived for

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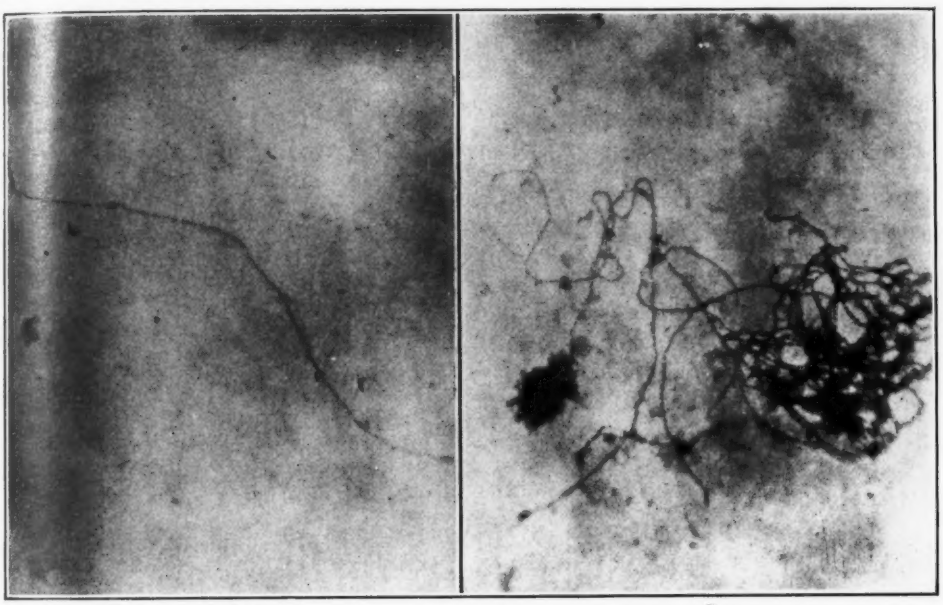
twenty-six hours, and the ninth mouse nine hours. A Type IX culture obtained from Case 1,408 was passed in the same manner for seven days. The first mouse lived thirty hours and the seventh died at the end of eleven hours.

A Type V culture isolated from Case 30,593 was passed through mice for four days. The colonies were "rough" when first isolated and remained so after the mouse passages. The culture continued to grow in chains. The culture from the heart of the fourth mouse failed to grow, though the mouse died. Because of the pressure of work the inoculation with the culture from the third mouse was not repeated and the series of inoculations continued.

A blood culture, Type VIII, isolated from Case 30,912, was streaked on agar plates to study the colonies. The edges were found to be irregular and



A. *B.*

Fig. 1.—*A.* Pneumococci growing in chains, from mouse's heart culture. *B.* Agglutination with homologous serum, of pneumococci growing in chains.

the centers depressed. Broth cultures had a puff-ball growth in the bottom of the flask with some stringlike growth extending upward to the top of the broth.

A blood culture isolated from Case 21,755 gave rough colonies and the broth cultures were very granular.

Fig. 1 shows the characteristic appearance of the chain formation which extends in some instances entirely across the field with occasional separate organisms and the typical tangled skeins found in agglutination tests with pneumococci in chains. It is noteworthy that the organisms are small with ill-defined capsules.

CLINICAL OBSERVATIONS

Our experience seems to show that where chainwise pneumococci were present, the cases were usually less severe than the average case. During

the period of the study there were 1,152 pneumonias in adults, of whom 49 presented pneumococci in chains, an incidence of 4 per cent, and 191 pneumonias in children, of whom 7 gave pneumococci in chains, or 3 per cent.

The occurrence of the chain formation was distributed by type, as indicated in Table I. Among 145 cases from which Pneumococcus Type V (Cooper), Subtype IIa of Avery, were recovered, 18 strains which grew as chains were isolated, or 14 per cent, and among 76 cases of Type VIII (Cooper), atypical III of Sugg, Gaspari, Fleming and Neill,[5] pneumococci in chains were isolated from 6 patients, or 8 per cent. Chain formation did not occur among 163 cases of lobar pneumonia due to Pneumococcus Type II.

The monthly distribution of this cultural appearance during the year is shown in Fig. 2. Apparently the number of strains having chain formation

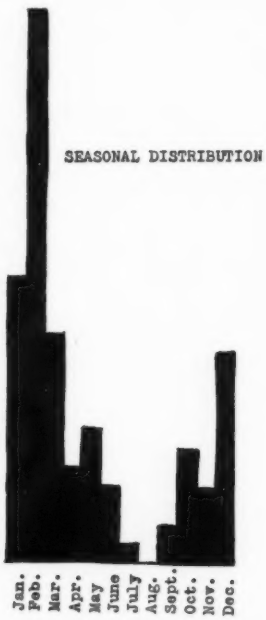


Fig. 2.—The occurrence of pneumococci growing in chains by months. The appearance of chains was most frequent when the cases were most numerous, and not in the summer time when the milder cases are more frequent. Each square is a case.

were in proportion to the number of cases, i.e., appeared most frequently when there were most cases. There were few chain-wise organisms encountered during the summer and fall months when the pneumonias are generally thought to be milder. The incidence commenced to fall when the cases were still numerous and severe in March.

It is significant that chain-wise formation was found in half the cases before the sixth day of illness. In some of the cases, our initial observation was later than that.

The only exception to the usual good prognostic correlation of the chain-wise appearance were the cases in which involvement with pneumococci of different type, which did not form chains (Case 4,915), or streptococcic invasion (Case 4,808) supervened, and in two of the five bacteriemic cases.

TABLE I

DISTRIBUTION BY TYPES OF PNEUMOCOCCUS CULTURES GROWING IN CHAINS; CULTURES ISOLATED FROM LOBAR PNEUMONIA CASES AND FROM OTHER RESPIRATORY INFECTIONS

I	9	XIX	8
II	0	XX	1
III	5	XXI	0
IV	5	XXII	0
V	22	XXIII	1
VI	4	XXIV	0
VII	2	XXV	0
VIII	6	XXVII	0
IX	2	XXVIII	2
X	1	XXIX	1
XI	0	XXX	0
XII	1	XXXI	0
XIII	2	XXXII	0
XIV	1	V and VII	1
XV	2	Neg. I-XXIII	11
XVI	1	Neg. I-XXXII	1
XVII	0		—
XVIII	3	Total	92

Note the frequency of Type V and the absence of Type II.

It will be seen from the summary of diagnoses (Table II) that in only a little more than two-thirds of the cases was the lung parenchyma invaded. Four of the 92 patients died, and we shall discuss these deaths in detail later.

TABLE II

SUMMARY OF DIAGNOSIS OF THE INFECTIONS OF PATIENTS FROM WHOM PNEUMOCOCCI IN CHAINS WERE ISOLATED

DIAGNOSIS	ADULTS	CHILDREN	INFANTS	TOTAL
Upper respiratory infections	7			7
Tonsillitis and peritonsillitis	1	1	1	3
Sinusitis and pharyngitis	1			1
Grippe	1			1
Bronchitis	3	3	3	9
Bronchopneumonia	2	5	2	9
Lobar pneumonia with tuberculosis	2			2
Lobar pneumonia	51	6	1	58
Pleurisy	1			1
Empyema	1			1
Total cases				92

The only cases, however, in which the organisms probably responsible for death were found in chains (because there was no change of pneumococcus type) are Cases 4,612 and 25,654. In Case 4,612 the organisms were in chains in the first blood culture; in the second blood culture the pneumococci grew in chains. Subsequent blood cultures were sterile. The patient received serum, agglutinins appeared and persisted. The final increase in pulse rate may have been associated with a terminal invasion but postmortem cultures were not taken. There was very marked dehydration. Case 25,654 had a positive blood culture of pneumococci in chains on admission, Sept. 24, 1932. Subsequent blood cultures were positive on some days and negative on other days. The cultures did not grow in chains in those made after Oct. 5, 1932.

There was an average of six colonies per cubic centimeter on the blood plates until the day of death, Oct. 13, 1932, when the plates were overgrown.

Only two cases from which a pneumococcus growing in chains was isolated, died among the 57 cases of lobar pneumonia. Table III gives a comparison between the mortality in lobar pneumonias in this group of cases and the lobar pneumonias in which the pneumococci did not grow in chains for the same period.

TABLE III

A COMPARISON OF THE MORTALITY IN LOBAR PNEUMONIA CASES CAUSED BY PNEUMOCOCCI OCCURRING IN CHAINS AND BY PNEUMOCOCCI NOT IN CHAINS

ADULTS																
	PNEUMOCOCCI IN CHAINS								PNEUMOCOCCI NOT IN CHAINS							
			NONSERUM			SERUM				NONSERUM			SERUM		
TYPE	TOTAL CASES	TYPE PER CENT	CASES	DEATHS	PER CENT	CASES	DEATHS	PER CENT	TOTAL CASES	CASES	DEATHS	PER CENT	CASES	DEATHS	PER CENT
I	6	1.6				6	0		370	54	16	30	316	50	16
										10*	8*	80*	96*	37*	38*
III	3	2.0	3	0					147	97	35	36	50	24	48
										22*	20*	91*	17*	13*	77*
IV	5	5.3	5	0					89	61	10	16	28	6	21
										7*	6*	85*	4*	4*	
V	16	13.2	10	1	10	6	1	16	105	75	16	21	30	9	30
			1*	1*		1*	1*			20*	9*	45*	13*	7*	53*
VI	1		1	0					22	18	4	22	4	2	50
										4*	3*	75*	1*	1*	
VII	2	1.7	2	0					117	92	21	23	25	2	8
										10*	8*	80*	1*		
VIII	6	7.9	4	0		2	0		70	48	9	19	22	3	14
										10*	5*	50*	7*	2*	28*
XII	1		1	0					14	14	3	21			
										5*	3*	60*			
XIII	2	10.0	1	0		1	0		18	16	3	19	2	1	50
													1*	1*	
XIV															
XV	1		1	0					6	6	2	33			
										2*	1*	50*			
XIX	1		1	0					15	15	8	53			
										7*	6*	86*			
XXVIII	1		1	0					4	4	1	25			
XXIX															
"x" group	4	1.7	4	0					236	236	51	22			
Mixed infections	2		Type I and Strep. Hem.—Bacteriemic—Died Type III and Strep. Hem.—Bacteriemic—Died												
Total	51	4.2	1113												

*The asterisk indicates bacteriemic cases.

A study of the cases of pneumonia shows that admission ratings, in accordance with our rating system, were rather high.[6] We have considered a rating below 50 as poor, from 50 to 70 fair, and above 70 as good. The temperatures were moderate. There was nothing to indicate unusual severity in the mode of pulse or respiration. It is noteworthy that none of the patients developed delirium and that anoxemia was not a severe feature. Oxygen was

TABLE IV

A COMPARISON OF THE MORTALITY IN PNEUMONIA CASES CAUSED BY PNEUMOCOCCI OCCURRING IN CHAINS AND BY PNEUMOCOCCI NOT IN CHAINS

CHILDREN															
	PNEUMOCOCCI IN CHAINS								PNEUMOCOCCI NOT IN CHAINS						
			NONSERUM			SERUM				NONSERUM			SERUM		
PNEUMOCOCCUS TYPE	TOTAL CASES	TYPE PER CENT	CASES	DEATHS	PER CENT	CASES	DEATHS	PER CENT	TOTAL CASES	CASES	DEATHS	PER CENT	CASES	DEATHS	PER CENT
I	1		1	0					49	24 1*	1	4	25 6*	2 1*	8 17*
III	1		1	0					22	21 1*	4 1*	19	1	0	
IV															
V	2		1	0		1	0		22	16 1*	0 0*		6 1*	0 0*	
VI															
VII															
VIII															
XII															
XIII															
XIV	1		1	0					67	53 3*	7 1*	13 33*	14	2	14
XV															
XIX	1		1	0					26	25	2	8	1	1	
XXVIII															
XXIX	1		1	0					5	5 1*	1 1*	20			
"X" group Mixed infection															
Total	7	3.4							191						

*The asterisk indicates bacteriemic cases.

required infrequently. The illness seemed to be shorter than usual. The blood counts were neither high nor extremely low, with the exception of one case (2,188) in which tuberculosis was associated, where the blood count was 7,000. There were no complications with the exception of otitis media, and an empyema which was present on admission. No attempt to give a percentage of the occurrence of the various symptoms is made because too little is known concerning the occurrence of such symptoms when pneumonias are studied and divided into their separate types.

FATAL CASES

The patients who died are worthy of special consideration. There were four deaths in this group of cases.

CASE 4,915.—A Pneumococcus Type III in chains was recovered from this patient's sputum on admission, and later agglutinins for Type III were demonstrable in his blood. This patient probably acquired a Type VII pneumococcus infection from a pneumonia patient with a severe Pneumococcus Type VII bacteriemia, who was placed in the bed next to him on the twentieth day of his illness. The temperature continued high and the patient became worse, dying on the twenty-sixth day. A Type VII pneumococcus was recovered from a lung suction culture on the day before death. Postmortem heart's blood cultures revealed a

Streptococcus hemolyticus. This case probably should not be counted as a death from the Pneumococcus Type III infection from which he had apparently recovered, having produced agglutinins, but a death from a virulent Type VII pneumococcus and a hemolytic streptococcus. It may be that a very severe clinical picture associated with a pneumococcus growing in chains should suggest that the condition possibly is due to a different organism which should be sought by further study.

CASE 4,612.—This patient had a Type V pneumococcus blood culture on admission. The organism grew in chains in the initial blood culture. The second blood culture was positive but the organisms were not in chains. All subsequent blood cultures were sterile. Marked dehydration was present throughout the illness and the patient died on the twelfth day of illness. Agglutinins for the invading organism developed and persisted. There were no pneumococci in the single sputum examination.

CASE 4,808.—Type I. This patient continued to have a high temperature, and six sputum examinations were made. In the sputum streptococci were found, as well as Type I pneumococci in chains. The results of the sputum examinations were as follows:

1. Neither pneumococci nor streptococci were recovered.
2. Streptococci.
3. No growth in mouse peritoneum.
4. Pn. Type I in chains.
5. Pn. Type I in chains.
6. Streptococcus.

This patient died on the fourteenth day and *Streptococcus hemolyticus* was recovered from the postmortem heart's blood. Two previous blood cultures taken on the third and tenth days of the illness were sterile. This patient received large amounts of Pneumococcus Type I serum without favorable result. The streptococci were probably of major importance, and account for the death.

CASE 25,654.—Type V. The blood cultures have been described (p. 1159). Fluid was obtained by chest tap on October 11. The patient died in pulmonary edema Oct. 13, 1932.

Seventeen of the patients received type specific serum. Seven of these were Type I, 7 Type V, 2 Type VIII, and one Type XIII. In other than Type I, experience with dosage is too limited to definitely determine the average amount required. From analogy with Type I the amounts which were efficient in all but three of the patients appear to be moderate as they were less than 50,000 units in eight instances. One hundred thousand units is our usual dose for Type I. Case 4,753, admitted on the second day, received 185,000 units of Type I serum. A gradual fall of temperature occurred on the seventh day. There had been a critical fall of pulse on the third day, and of temperature on the fourth day, the former after 25,000 units and the latter after 55,000 units. The defervescence did not last and the agglutinins did not persist in the blood. A small amount of fluid was removed. Empyema did not develop.

SUMMARY

1. The phenomenon of chain formation in pneumococci and its association with "rough" colonies and lowered virulence for mice is described.

2. Pneumococci growing in chains occurred in 4.2 per cent of the adult pneumococcus pneumonias, and in 3.4 per cent of the childhood pneumonias.

It occurred in 14 per cent of all Type V pneumococcus pneumonias, in 8 per cent of all Type VIII pneumococcus pneumonias and in 5 per cent of all Type XIII cases.

3. The occurrence of chain-wise growth of pneumococci in severely ill patients suggests the possibility that the infection may be due to some other organism, either a streptococcus or a pneumococcus of another type. Chain-wise appearance occurring in pneumococci recovered from the blood should not be a deterrent to the use of available specific serum as the appearance of chain formation may be due to a partial protection in the blood. This protection may become exhausted.

4. The cases for the most part appear to be less severe than pneumonias due to pneumococci which do not form chains.

The authors record their appreciation of the criticism and suggestions of Dr. William H. Park and Georgia Cooper.

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OBSERVATIONS ON OXYGEN THERAPY*

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WITHIN recent years oxygen therapy has become increasingly popular in clinical practice, and has proved of definite value in helping certain patients. But the question has arisen whether its more widespread use is entirely deserved. The problem can be approached in one of two ways: (1) by making a statistical summary of its effects based on clinical impression; and (2) by determining its action on the various functions of the body in controlled experiments on a few subjects, and from such determinations estimating the possible benefit in various types of illnesses. This is not the place to enter into the relative merits or limitations of the two types of approach. The recent studies on oxygen therapy with which the author was associated[1, 2, 3, 4] were based on the latter method, and this method of approach will be followed in this communication.

It is definitely established that oxygen is essential for the life and well-being of all body cells. Its removal or diminution is detrimental to all cells, affecting some more rapidly than others. The cells contain practically no store of oxygen and are, therefore, entirely dependent on the oxygen brought to them in the capillaries. In many diseases an interference with the oxygen supply to the tissues occurs, a condition known as anoxemia, or more accurately, anoxia. Anoxia may be localized to one region or may be general throughout the body. It may be mild, moderate, or severe in degree; it may be acute, subacute or chronic in time; and it may be one of several varieties. Barcroft[5] recognized three forms of anoxia: (1) The anoxic form in which the oxygen want of the tissues is due to an imperfect aeration of the arterial blood so that the blood reaching the capillaries does not carry oxygen up to its full capacity. (2) The anemic form in which the arterial blood carries its full capacity of oxygen, but the oxygen carriers are decreased in amount either because the hemoglobin is diminished in concentration or is changed in character. (3) The stagnant form, in which the arterial blood is saturated with oxygen to capacity and the oxygen carriers are adequate in amount, but the difficulty is in the slow rate at which blood passes through the capillaries. Recently Peters and Van Slyke[6] have added a fourth form, the histotoxic, in which the cells themselves have difficulty in utilizing the available oxygen. Of course, in many diseases two or more forms of oxygen want may be combined. This division of oxygen want into various forms and degrees is of more than academic importance, because, as will be shown in a moment, a consideration of these facts will determine the rational application of oxygen therapy.

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What can oxygen therapy do? It is quite obvious that increasing the proportion of oxygen in the air which the patient inhales from one-fifth, normally present at sea level, to one-half of the total air breathed will increase the oxygen content in his alveoli, and therefore, the quantity of oxygen passing into the blood. The amount of oxygen that can go into physical solution in the blood is very small; practically all of it combines in a loose chemical form with the hemoglobin. Obviously, when the hemoglobin is saturated with oxygen very little more can be added. It is, therefore, self-evident that when the oxygen want is of the anemic, stagnant, or histotoxic forms no improvement from oxygen therapy may be expected. In carbon monoxide poisoning, oxygen and carbon monoxide can be viewed as competitors for hemoglobin, and increasing the available oxygen will drive off the carbon monoxide more rapidly. Oxygen therapy, therefore, may be of value in this condition.

Oxygen therapy is primarily of value when the arterial blood does not contain oxygen up to its capacity. But it may be used also where it is anticipated that the arterial blood will become unsaturated because the demands of the tissues for oxygen are expected to increase. This happens especially in postoperative states. In such conditions oxygen therapy might be considered as a prophylactic measure.

Oxygen therapy may not always raise the oxygen content of the arterial blood when the blood is not fully saturated with oxygen. Let us investigate this point further. Incomplete saturation of the arterial blood with oxygen may be due to several categories of causes. One cause is a short circuit of some of the venous blood which avoids air-containing alveoli. Some of the blood returning to the arteries remains in its "venous" state. Congenital heart disease, pneumonia, and atelectasis of the lungs may give rise to these shunts. It is obvious that adding more oxygen to the air-containing alveoli will have little effect on the oxygen content of the arterial blood since the oxygen cannot come in contact with the blood that has been short circuited. It is for this reason that occasional cases of pneumonia have been reported in which the arterial unsaturation is not changed by residence in a high oxygen atmosphere.[4, 7, 8]

In the other types of arterial unsaturation, oxygen therapy may be expected to increase the oxygen content of the arterial blood. In the type of anoxia due to an excessively rapid flow of blood through the lung capillaries, such as may occur in hyperthyroidism, disseminated pulmonary embolism, pneumonia and severe exercise, oxygen therapy will accelerate the rate at which oxygen can enter the blood and so compensate for the rapidity of the blood flow. In the type of anoxia due to rapid shallow breathing, oxygen therapy, best combined with CO_2, will break the vicious cycle of shallow breathing which the oxygen want itself aggravates. In the type of anoxia due to the presence of excessive fluid in the lung alveolar walls and spaces, which follows passive congestion, inflammation, infarction and the irritant effect of war gases and foreign bodies in the lung, oxygen therapy enhances the force which drives oxygen into the blood, and therefore, tends to overcome the increased resistance offered to the passage of oxygen. In the type of anoxia due to

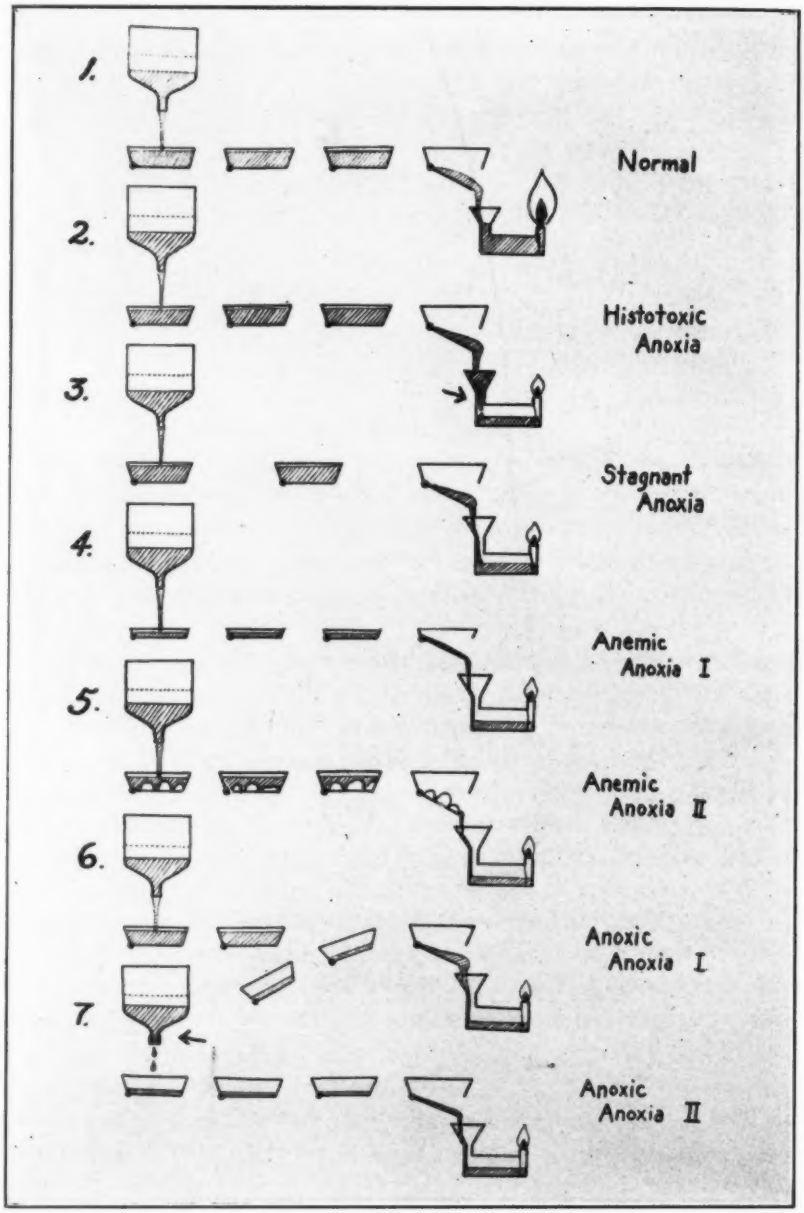

Fig. 1.—A diagrammatic representation of the processes of internal and external respiration in various types of oxygen want, which shows the mechanism of action of oxygen therapy.

In the first diagram is shown the condition normally existing; at the left, the oxygen is being poured into the blood stream, represented by little boats from the lung reservoir (external respiration). These boats, as shown by the shaded areas, are filled almost to capacity. These boats move to the right (transportation). At the right, the oxygen is shown being poured out from the blood into the tissue cells which keep the "flame of life" burning vigorously (internal respiration). Increasing the level of oxygen in the lung reservoir to the dotted line, as occurs in oxygen therapy, has little effect on the oxygen content of the boats already fully loaded.

In the second diagram is shown the condition in histotoxic oxygen want. The only difference between this condition and the normal lies in the fact that the tissue cells cannot utilize their oxygen so readily. This is indicated by a constriction in the stem of the funnel leading to the "lamp." As a consequence the "flame of life" burns less vigorously, or may even snuff out. Raising the oxygen level in the lung reservoir by oxygen therapy, can have little effect in this condition since the boats are already almost full.

obstruction of the air passages or decrease in lung expansibility, oxygen therapy will tend to compensate for the poor aeration of the alveoli.

Cyanosis has been used by clinicians as an index of a low oxygen content in the arterial blood. The recent work of Lundsgaard and Van Slyke[9] in particular has shown that this is an imperfect index. Cyanosis is a color change due to the presence of a definite quantity of nonoxygen carrying (reduced) hemoglobin in the capillaries beneath the skin. The reduced hemoglobin in the capillary blood is roughly the average of that in the arterial and venous blood of the region. If the circulation be sluggish, either locally or throughout the body because of heart failure or shock, then the greater removal of oxygen by the tissues will increase the reduced hemoglobin in the capillary blood above normal. If, for this reason, the reduced hemoglobin of the capillaries exceeds 5 gm. per cent, cyanosis will appear. Cyanosis, therefore, will appear more readily than normal in polycythemic individuals, and less readily than normal in the anemic. It will occur more readily than normal when the subcutaneous capillaries are engorged. It is well known among clinicians that the ashen-gray color associated with general anemia or local pallor is more significant than the slight cyanosis of the florid polycythemic individual. It will be seen from this discussion that cyanosis does not necessarily mean anoxia of the anoxic form, and, therefore, without further evidence cannot be taken as a criterion for the use of oxygen therapy.

Recent observations have shown that oxygen therapy has certain side actions which may be of value, namely, it tends to slow the rate of respiration and the minute volume of air breathed. Accompanying this there is an increase in the alkali reserve and carbon dioxide content of the blood. There is a tendency for the heart to slow (except in auricular fibrillation), and there is a drop in temperature of from ½ to 1° C. in patients with pneumonia according to Boothby.[10] The effects on respiration and the heart have been shown to occur not only in patients with oxygen want but in normal subjects as well.[3, 11] The manner in

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In the third diagram is shown the condition existing when the oxygen want is due to a sluggish circulation. The slow movement of blood is represented by a reduced number of boats. The delivery of oxygen to the tissue cells is decreased, hence their oxygen supply falls and again the "flame of life" decreases in intensity. Raising the oxygen level in the lung reservoir by oxygen therapy can have little effect in this condition since the boats are already almost full.

The fourth diagram represents the condition when the oxygen want is due to anemia. The small size of the boats represents the reduced capacity of the blood for carrying oxygen. The amount of oxygen reaching the tissue cells will, therefore, be decreased and the "flame" will again dwindle. Raising the oxygen level in the lung reservoir will not remedy the condition since the boats are already almost full.

The fifth diagram represents the condition when the oxygen want is due to carbon-monoxide poisoning. The reduced capacity of the blood for carrying oxygen, due to the formation of carbon-monoxide hemoglobin, is represented by the "stones" in the boats. Again the "flame of life" is lessened. Oxygen therapy, which raises the oxygen level in the lung reservoir, is of little avail except so far as it can displace carbon monoxide and dislodge some of the "stone ballast" in the boats.

The sixth diagram represents the condition when the oxygen want is due to a decreased oxygen content of the blood in the arteries, brought about by an admixture of "venous" blood. Some of the boats never pass by the lung reservoir, so that some of them are not fully laden when they reach the tissue cells. The oxygen delivery to the tissue cells is, therefore, decreased and the "flame of life" dwindles. Raising the level of oxygen in the lung reservoir will not remedy the condition because some of the partially empty boats are shunted and never reach the lung reservoir.

The last diagram shows the condition when the oxygen want is also due to a decreased oxygen content of the blood in the arteries. In this case, however, the incomplete filling of the boats is caused by an interference with the exchange of oxygen in the lungs. This is represented by a constriction at the mouth of the lung reservoir. The delivery of oxygen to the tissue cells is reduced because of the small loads carried by the boats and the "flame of life" dwindles. Obviously, increasing the level of oxygen in the lung reservoir by oxygen therapy will tend to overcome the constriction and fill the boats more completely. This is the condition in which oxygen therapy has possibilities of usefulness as discussed in the text.

which these side actions are produced is still unsolved. Whether it is some environmental factor in the oxygen chamber or a reflex set up by the increased oxygen content of the alveoli remains undetermined. However, both the slowing and diminution of respiration and the slowing of the heart are beneficial factors. The slowed heart beats more efficiently, and the load of work on the patient is decreased when respiratory effort is lessened. A similar action follows the lowering of body temperature. However, if these side actions were the only benefits to be derived from oxygen therapy they would hardly seem to warrant the trouble and expense involved in the use of oxygen.

From the foregoing analysis, it is clear that if oxygen therapy is to be used, it should be employed to increase the arterial oxygen saturation of those forms of arterial oxygen want where previous observations have shown that this can be accomplished. Oxygen therapy should be used where there is the possibility that the oxygen want contributes to the illness of the patient and where the likelihood exists that benefit will follow relief of the oxygen want. Therefore, oxygen therapy is most valuable in the acute and severe forms of oxygen want where the deleterious results of oxygen want rapidly outdistance bodily adaptations. There is less reason to use oxygen therapy in the chronic and mild forms of oxygen want where bodily adaptations have apparently compensated for the anoxia. In other words, oxygen therapy when used at all should be a temporary measure to alleviate acute, severe, generalized anoxic anoxia, where it is thought that restoring the arterial oxygen saturation will benefit the patient and check a downward clinical course. Oxygen therapy in no way acts on the underlying disease but only on the syndrome which follows anoxia. It should not be substituted for the ordinary armamentarium of the physician but be employed as an adjunct to the other forms of treatment. When given, oxygen therapy should be continuous until the desired effect is obtained, and should be maintained until the indications for its use no longer exist.

On this basis, oxygen therapy is especially valuable in postoperative conditions where rapid shallow breathing is present or where pneumonia seems imminent or has developed. It is, of course, valuable in other forms of pneumonia, but not in all cases. In cardiac patients it is valuable when there is evidence of severe passive congestion of the lungs and particularly in acute pulmonary edema. It can be of little use when these are absent and only the stagnant form of oxygen want exists. There is no evidence that it can benefit angina pectoris or long-standing cardiac edema unless these conditions are accompanied by severe pulmonary edema or stasis, except for the psychic effect on the patient which any elaborate therapeutic procedure can produce. Since it has no effect on the disease itself, oxygen therapy may fail even when the indications for its use are optimal and where relief of arterial oxygen unsaturation is attained.

The definite important place which oxygen therapy has in clinical practice will doubtless expand with further careful studies.

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THE DIETARY TREATMENT OF UNDERNUTRITION*

II. Effect of the Gain in Weight on Carbohydrate Tolerance

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THE use of insulin to increase the weight of individuals who are underweight, seems to us unnecessary, except for the occasional patient who lacks the appetite to eat a high calorie diet. In the first paper of this series[1] we showed that on a high calorie diet the gain in weight over a period of six weeks was as great as the gains reported with the use of insulin,[2, 3] except for one case reported by Nahum and Himwich.[4] There are certain disadvantages to the use of insulin. Aside from the inconvenience there is the possibility of hypoglycemia. Another effect, reported by Paul, Clark, and Gibson[5] is that following the withdrawal of insulin in nondiabetic patients, there occurred both hyperglycemia and glycosuria. This was observed in all six of their patients. On three of the patients glucose tolerance curves were done and showed a definitely diminished tolerance for carbohydrate. In one patient, followed for eight days after the discontinuance of insulin, the tolerance had still not returned to normal.

We are reporting the effect of a high calorie diet, which was accompanied by a definite gain of weight, on the carbohydrate tolerance of the six individuals observed. The subjects were third-year medical students. The diet contained approximately 430 gm. of carbohydrate, 175 gm. of protein, and 240 gm. of fat. The total calories ingested averaged about 4,500, although as high as 5,500 was taken for the last week by Cases 7 and 8. The carbohydrate tolerance was measured by glucose tolerance curves. These were done the morning before the diet was begun and the morning after the dietary period was finished. In each case 100 gm. of glucose was given by mouth.

During the period of the diet the students continued at their work and in no way curtailed their ordinary activities. The patients in Cases 7, 8, 9,

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and 10 had always been underweight. The patients in Cases 11 and 12 had lost weight during the preceding year. The subjects were normal in all other respects.

RESULTS AND DISCUSSION

Table I shows the weekly gain in weight in the 6 patients. The results are similar to those observed in the first group of patients.[1] The greatest gain in weight always occurred in the first week. The patients in Cases 11 and 12 were taking their final examinations during the last three weeks of the diet and did not eat the entire diet at this time. This is reflected in their weight charts.

TABLE I

POUNDS GAINED WEEKLY ON DIET

CASE	WEEKS—POUNDS GAINED							TOTAL	WEEKS
	1	2	3	4	5	6	7		
7	5.0	3.0	3.5	3.5	3.0	2.5		20.5	6
8	4.0	3.75	3.0	3.25	2.5	1.75		18.25	6
9	4.75	2.5	1.5	1.5	2.0	2.5	2.5	17.25	7
10	5.5	3.25	3.75	2.25	2.5	–		17.25	5
11	7.0	2.0	5.0	0.0	1.0	0		15.0	6
12	5.5	0.0	4.0	1.5	1.5	0		12.5	6
Average	5.3	2.41	3.46	2.0	2.0	1.35		16.8	

TABLE II

GLUCOSE TOLERANCE CURVES

CASE	BEFORE DIET BLOOD SUGAR MG.					POUNDS WT. GAINED	AFTER DIET BLOOD SUGAR MG.				
	FAST	½ HR.	1 HR.	2 HR.	3 HR.		FAST	½ HR.	1 HR.	2 HR.	3 HR.
7	93	138	132	102	93	20.5	83	102	111	106	84
8	101	114	118	95	70	18.25	89	160	133	100	73
9	87	152	154	116	78	17.25	76	105	133	117	81
10	80	133	105	76	69	17.25	80	117	129	105	72
11	71	86	125	105	86	15.0	101	121	104		
12	76	117	166	125	95	12.5	75	81	103	72	

Table II shows the glucose tolerance curves on these subjects before and after the dietary period. These are all within normal limits. The average of the group is shown graphically in Chart 1. The second curve should be compared with the curve reported by Paul, Clark and Gibson[5] following the discontinuance of insulin. Individually the curves show that the height of the hyperglycemia after the dietary period was less in Cases 7, 9, 10, and 12, practically unchanged in 11. In the patient in Case 8 at the ½-, 1-, and 2-hour periods the hyperglycemia was greater than before the diet, although always within normal limits.

The students were weighed twice daily, before breakfast and after supper. Of the weight gained during the day, an average of 4 pounds was lost overnight. The true weight was that taken before breakfast. The constancy of this difference is shown graphically in Chart 2, which records the daily weights on Case 7. This is typical of the other five cases.

The ability of an individual to gain weight depends on his taking in more energy than he puts out. That this can be accomplished by means of a high calorie diet is shown in this group of subjects and in the group previously

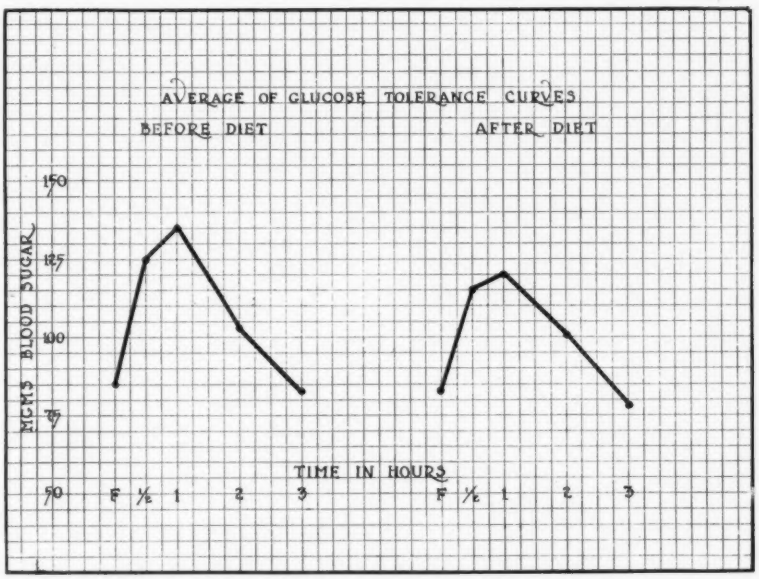

Chart 1.

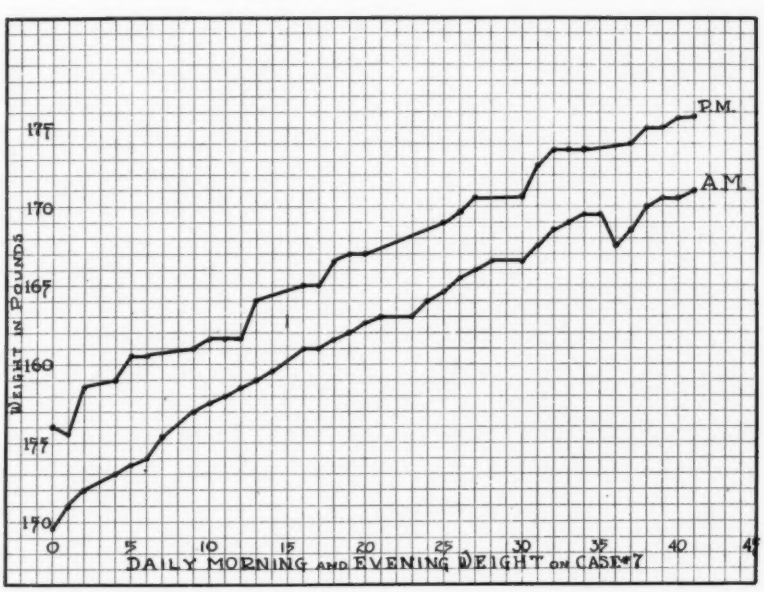

Chart 2.

reported.[1] The chief effect of insulin in undernutrition is its ability to stimulate the appetite. This makes it easier for the patient to eat a large amount of food. The gain in weight is due to the increased caloric intake. If the patient does not eat, insulin will not increase the weight. We had under

treatment in the hospital a patient twenty-three years of age to whom insulin was given 3 times daily before meals, ten units before breakfast, 15 before lunch, and 15 before supper. The patient had hypoglycemic reactions almost daily, but an increased appetite was not experienced. She refused to eat the entire diet and during a period of three weeks gained exactly 4 pounds.

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HIGH CALORIE DIET

BREAKFAST	CHO.	PRO.	FAT
1 glass orange juice with 2 tsps. sugar	34	2	0
2 glasses milk with 2 tsps. lactose	29	20	16
1 oz. cereal with 2 tsps. sugar	33	3	0
100 gm. 20 per cent cream	2	2	20
2 slices toast	36	6	0
1 oz. butter	0	0	25
2 eggs	0	12	12
2 tbs. jam or jelly	25	0	0
NOON			
5 oz. meat	0	30	30
5 oz. potato	30	3	0
2 fresh vegs.	12	2	0
2 glasses milk with 2 tsps. lactose	29	20	16
2 slices toast	36	6	0
1 oz. butter (or 2 tbs. mayonnaise)	0	0	25
100 gm. dessert with 2 tbs. cream whipped	20	7	10
NIGHT			
1 glass tomato juice	5	1	0
5 oz. meat	0	30	30
5 oz. potato	30	3	0
2 fresh vegs.	12	2	0
1 glass milk	12	10	8
1 slice toast	18	3	0
1 oz. butter	0	0	25
1 baked apple with 2 tsps. sugar	25	3	0
100 gm. (20 per cent) cream	2	2	20
NIGHT FEEDING			
1 glass milk	12	10	8
2 Uneeda biscuits	10	1	1
Caloric value 4,574	423	177	246

When one considers that an impaired tolerance for carbohydrate may follow the use of insulin in nondiabetic individuals, and since a satisfactory gain of weight will follow the ingestion of a high calorie diet, it seems to us that the indiscriminate use of insulin in undernutrition is inadvisable.

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GAUGING THE DOSE OF INSULIN

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SINCE its introduction in 1922, insulin, one of the few specifics in medicine, has found wide acceptance. Unlike other specifics (morphine, quinine, the arsenicals, etc.), there is no approximately "standard dose" for insulin. Necessarily the insulin requirement varies with the individual patient, depending upon the indeterminate insulin secretion within his own body and the glucose equivalent of his diet. Hitherto achievement of adequate insulin dosage has been a tedious process. One of the standard usages defines that the patient be placed upon an almost starvation diet until the urine is sugar-free, that the food intake be gradually increased, and finally that he be given a number of units of insulin equal to one-half the amount of glucose, measured in grams, in the twenty-four-hour urine. As the diet is further increased insulin is further readjusted depending upon the urinary glucose content. If the patient be in diabetic coma, a standard plan calls for the administration of a definite number of units of insulin, usually between 20 and 40, each hour until the urine is sugar-free.

At best the methods are cumbersome. They fail to take into sufficient account the varying renal threshold for glucose.[1] Not infrequently the patient shows no glycosuria although the blood sugar is well above 250 mg. per cent. On the other hand an insulin reaction may be precipitated by failure to consider residual sugar in the bladder urine; or by failure to remember that glycosuria usually continues below the glycemic level at which it began.

The ideal treatment of diabetes is that which insures a twenty-four-hour blood sugar curve analogous to that found in the nondiabetic. In addition to restoring the disturbed osmotic balance of the blood upset by the increase in blood sugar, maintenance of a normal blood sugar curve theoretically insures complete utilization of all available carbohydrate. It is common knowledge that the continuously aglycosuric diabetic with a normal blood sugar level develops a spontaneous increase in tolerance not shown by the intermittently glycosuric diabetic. Since bodily resistance is at least as great in the adequately controlled diabetic as in the nondiabetic, intercurrent infection and subsequent debility are rare.

With these considerations in mind I have found the following empiric method of gauging the insulin requirement of individual patients useful. It must be mentioned that the method has no logical basis in fact, unless it be that clinical experience has amply confirmed its complete safety and relative value. It is assumed that all of the glucose present in the body is equally distributed in all the tissues (regardless of the possible actuality); if the

blood of a 70 kg. patient contains 500 mg. per cent glucose (0.5 gm. per 100 c.c.), then the total glucose in the entire body may be assumed to be represented by the formula

$$\frac{70{,}000 \text{ (gm. body weight)} \times 0.5}{100} = 350 \text{ gm.}^2$$

Since the normal blood glucose (and body tissue glucose?) is roughly 100 mg. per cent (0.1 gm.), modification of the formula enables determination of the glucose present as excess:

$$\frac{70{,}000 \times 0.5\text{–}0.1}{100} = 280 \text{ gm. representing the amount of glucose which}$$

must be either burned, excreted, or synthesized into glycogen to restore a normal blood sugar level! A number of units of insulin equal to one-half the excess glucose in grams is assumed to be required to utilize the excess glucose; as a safety measure only four-fifths the estimated dose is given. Hence in the hypothetical case here cited, 112 units $\left(\frac{280}{2} \times \frac{4}{5}\right)$ of insulin may be given the patient.

The formula may be simplified for routine use as follows:

$$\frac{\text{Body weight in pounds}}{6} = \text{the number of units of insulin to be given}$$

for each 100 mg. per cent the blood sugar is above the normal glycemic level (100 mg. per cent). Applying this formula to the 154-pound (70 kg.) patient with a blood sugar of 500 mg. per cent, it is evident that 104 units $\left(\frac{154}{6} \times 4\right)$ of insulin will be required. As a matter of actual fact it has been my usual experience that such an amount of insulin will reduce the blood sugar only four-fifths (the safety factor) of the expected reduction; however, such a procedure enables avoidance of an insulin reaction. It has been found by experience as well as by unpublished studies that this change may usually be expected to take place within from two to four hours.

EXAMPLES

CASE 1.—L. H., female, aged fifty, weight 154 pounds. Blood sugar at time of injection of 70 units of insulin: 300 mg. per cent. Calculated blood sugar reduction: about 200 mg. per cent. Blood sugar two hours after insulin injection: 100 mg. per cent. Actual blood sugar reduction: 200 mg. per cent.

CASE 2.—F. E., female, aged fifty, weight 176 pounds. Blood sugar at time of injection of 120 units of insulin: 471 mg. per cent. Calculated reduction: about 300 mg. per cent. Blood sugar two hours after insulin injection: 131 mg. per cent. Actual reduction 340 mg. per cent.

CASE 3.—J. D., male, aged thirteen, weight 108 pounds. Blood sugar at time of injection of 120 units of insulin: 1,000 mg. per cent. Blood sugar two hours later: 550 mg. per cent. An additional 80 units of insulin was given. Calculated total reduction: about 820 mg. per cent. Blood sugar two hours after second insulin injection: 133 mg. per cent. Actual reduction: 867 mg. per cent. Some hours later the patient had to be given, at intervals, two small doses of adrenalin for the relief of clinical insulin shock. The following morning, sixteen hours after the last blood analysis, the blood sugar was 200 mg. per cent. The

importance of prompt and energetic treatment in diabetic coma is emphasized by the finding of a blood nonprotein nitrogen at this time of 42 mg. per cent; one may only conjecture how high it may have been during the intense acidosis accompanying the very high blood sugar. In another patient treated according to the same principles, the blood nonprotein nitrogen fell from 75 mg. per cent to 64 mg. per cent in ten hours and to 45 mg. per cent in three days; during the first ten hours the blood sugar fell from 900 mg. per cent to the normal level.

CASE 4.—L. M., female, aged fifty-three, profound coma, estimated weight: 75 or 80 pounds. Given 50 units of insulin on admission to the hospital. Blood sugar just before injection: 1,100 mg. per cent; blood nonprotein nitrogen: 119 mg. per cent. Complicating bronchopneumonia. As soon as the blood sugar report was obtained, the patient was given an additional 100 units of insulin. Calculated total reduction: about 830 mg. per cent. It was not possible to obtain another blood sugar determination until ten hours after the second insulin injection; at that time the blood sugar was 117 mg. per cent, the nonprotein nitrogen, 200 mg. per cent. Actual blood sugar reduction: 983 mg. per cent. Subsequent autopsy revealed an extensive nephrotic process in both kidneys as well as the bronchopneumonia noted at entry to the hospital.

Comment: The body weight formula described above (last formula) would have called for 160 units of insulin in Case 3 instead of the 200 actually given, and in Case 4 for 130 units instead of 150. Hence, it is felt that the margin of safety allowed by the revised formula is sufficiently great to permit its employment by the general practitioner. No generalized urticarial eruption or other anaphylactic manifestation was observed after the administration of these large amounts of insulin. Several years ago the former usually followed the administration of as much as 100 units of insulin.

Note: Patients are carefully watched for the appearance of symptoms of insulin shock; if glucose or orange juice cannot be given by mouth adrenalin hydrochloride (4 to 10 minims) hypodermically may be depended upon to relieve such symptoms without waiting for the report of blood sugar analysis. If necessary it may be followed by the intravenous or intracardiac injection of 50 per cent glucose (10 to 20 c.c.).

Note: In management of the postoperative diabetic the blood sugar is determined two hours after the patient's return from the operating room; the insulin requirement is calculated as above and further modified as food is allowed. By the use of this method we have successfully carried one patient with a previously uncontrolled diabetes through an immediate laparotomy for acute suppurative appendicitis. Neither acidosis nor insulin reactions marred a smooth postoperative course; no intravenous glucose was necessary at any time.

Contraindications: Patients with known myocardial damage. Since we give no insulin by preference, a very little if absolutely required, to such patients, untoward reactions have not been observed.

INSULIN DOSAGE IN ROUTINE DIABETIC MANAGEMENT

If diet alone proves insufficient to render the patient aglycosuric and the blood sugar normal two hours after a meal (preferably breakfast), the patient is placed in the hospital and immediately given the diet calculated as necessary for his maintenance upon discharge. From the blood sugar deter-

mined at entry the insulin required for the metabolism of the endogenous glucose is determined according to the formula described above. Further, it is assumed that one unit of insulin will metabolize four grams of exogenous glucose;[3] in the same manner as before only four-fifths the estimated dose is given to allow a safety factor; this amount may be easily ascertained by giving a number of units of insulin equal to one-fifth of the available glucose in the diet as measured in grams. Exogenous available glucose is calculated in the usual manner: 100 per cent of the carbohydrate plus 60 per cent (actually only 58 per cent) of the protein plus 10 per cent of the fat. The insulin is divided into one, two, or three doses depending upon the total requirement; the largest dose is given before breakfast, the smallest before lunch, and the intermediate dose before supper. Two hours after breakfast on the third day of this regime the blood sugar is again determined to permit further insulin regulation in preparation of the patient for discharge from the hospital; usually it is between 90 and 150 mg. per cent. If at the former level, insulin dosage is reduced; if higher than 150 mg. per cent, insulin dosage is readjusted on the basis of the endogenous glucose reflected in the blood sugar level. Ordinarily the insulin readjustment made at this time is sufficient to carry the patient for several weeks until mild insulin reactions indicate the need for further readjustment. This method permits the patient to receive intensive diabetic instruction in the hospital and return to his gainful occupation in from four to seven days.

Essentially the same plan is used in the after-care of the coma patient except that instead of basing the insulin dosage for the endogenous glucose on the blood sugar either on admission to the hospital or after its reduction by a massive dose of insulin, it is arbitrarily assumed that the endogenous glucose excess is represented by a blood sugar of 300 mg. per cent.

If for any reason the diet formula needs to be changed (for instance if the carbohydrate in the diet is to be increased in preparation for operation) insulin is readjusted according to the difference in the exogenous glucose supplied by the two diets.

EXAMPLES

CASE 5.—M. R., female, aged thirteen, weight 65 pounds. Blood sugar on admission to the hospital: 400 mg. per cent; insulin required for metabolism of the endogenous glucose as calculated: 33 units. Insulin required for metabolism of exogenous glucose supplied by diet of protein 40 gm., fat 60 gm., carbohydrate 100 gm., available glucose 138 gm.: 28 units. Total insulin requirement: 61 units. Sixty units of insulin, 30 before breakfast, 10 before lunch, and 20 before supper were administered. Two hours after breakfast on the second day of this regime the blood sugar was 181 mg. per cent. The insulin was increased by 20 units instead of by the calculated 10 units and there were questionable mild insulin reactions. On the fifth day of this regime the diet was changed to protein 40 gm., fat 75 gm., carbohydrate 80 gm., available glucose 112 gm., a decrease of 26 gm. of available glucose calling for a reduction of 8 units of insulin (when small amounts of insulin only are involved the customary four-fifths reduction is usually omitted). On the third day of the new regime during which she received 30 units of insulin before breakfast, 15 before lunch, and 25 before supper, the blood sugar two hours after breakfast was 154 mg. per cent. During the course of the next few days mild insulin reactions indicating a considerable increase in tolerance occurred and the insulin was further reduced to 25 units before breakfast, 10 before lunch and 20 before supper. Two months after dischage from the hospital this patient

had gained 20 pounds in weight and had been able to reduce the insulin to 20 units before breakfast, none before lunch and 15 before supper. Nine months after discharge she had gained an additional 10 pounds in weight and had further reduced the insulin to 15 units before breakfast and supper.

CASE 6.—C. R., female, aged eleven, weight 62 pounds. Admitted to the hospital in coma with a blood sugar of 666 mg. per cent. Treatment seemed ineffective and on the second hospital day I was asked to see the patient. She was semistuporous and quite weak; there was no fever or infection. At that time her blood sugar was 375 mg. per cent; insulin required for metabolism of the endogenous glucose: 27 units. I changed the diet of protein 35 gm., fat 50 gm., carbohydrate 80 gm., available glucose 106 gm. with 15 units of insulin before breakfast, 10 before lunch, and 15 before supper to protein 35 gm., fat 90 gm., carbohydrate 80 gm., available glucose 110 gm. Hence no change in the insulin required for metabolism of endogenous glucose was indicated. Uncertain of the "hangover" effect of certain questionable treatment earlier employed, the insulin was increased only 15 units, a little more than half of that calculated, so that the patient now received 20 units before breakfast, 15 before lunch and 20 before supper. On the third morning of this regime the blood sugar two hours after breakfast was 138 mg. per cent and on the seventh morning at the same time it was 125 mg. per cent.

CASE 7.—E. R., male, aged thirty-two, weight 128 pounds. Blood sugar on admission: 500 mg. per cent; calculated insulin requirement: 84 units. Actually he was given 100 units and suffered an insulin reaction so mild that only one ounce of orange juice was required to relieve it. Unfortunately it was not possible to obtain a blood sugar determination until twelve hours after the insulin administration; at that time it was 140 mg. per cent. Assuming that the blood sugar would rise to about 300 mg. per cent spontaneously, 42 units of insulin would be required for its control. The diet of protein 70 gm., fat 160 gm., carbohydrate 80 gm., available glucose 138 gm., required 28 units of insulin for metabolism of the exogenous glucose. The total insulin of 70 units was given, 30 units before breakfast, 15 before lunch and 25 before supper. (To simplify the problem two intermediate days with a relatively higher carbohydrate allowance have been omitted from the discussion.) On the third day of the regime outlined above the fasting blood sugar was 190 mg. per cent while two hours after breakfast it was 125 mg. per cent. The latter figure indicates that the patient was adequately controlled; to lower the fasting blood sugar it would have been necessary to give a midnight dose of insulin, a procedure which we dislike because of the inconvenience caused the patient. The patient was discharged from the hospital on the seventh day. Three months after discharge he had gained 15 pounds in weight and reduced his insulin by 5 units, to 30 before breakfast, 15 before lunch, and 20 before supper.

Note: In patients running an irregular fever less than the total optimal dose of insulin as calculated is given in order to minimize the possibility of an insulin reaction when the fever reaches a low point. However, in the case of an infection with a continued fever more insulin is usually required. I am unable to propose any definite rules for the diabetic management of such patients inasmuch as their insulin requirement varies from hour to hour.

Contraindications: As above mentioned. In patients with severe myocardial damage no insulin is given even though the blood sugar be as high as 300 mg. per cent. If acidosis supervenes insulin may be given cautiously in doses of 5 or 10 units at a time. Onset of anginal pain is considered an absolute contraindication to its further use.

SUMMARY AND CONCLUSIONS

1. The blood sugar of a diabetic patient in coma or of one presenting a high blood sugar may be safely and quickly reduced by giving a number of

units of insulin equal to one-sixth the body weight in pounds for each 100 mg. per cent the blood sugar is above the normal glycemic level (100 mg. per cent). If the physician cares to use larger immediate doses of insulin, carefully watching the patient at the time, he may give one unit of insulin per kilogram body weight for each 200 mg. per cent that it is desired to lower the blood sugar. While the ordinary supportive measures such as saline hypodermoclysis and cardiac stimulants are given according to indications, glucose, sodium lactate, etc., are not ordinarily employed in the treatment of the coma patient. The rapidity with which the symptoms may be controlled makes the latter medication superfluous.

2. Routine regulation of the diabetic patient presenting no complications may be quickly and safely attained by giving a number of units of insulin determined as above for the metabolism of the endogenous glucose plus a number of units of insulin equal to one-fifth of the available glucose in the diet as measured in grams. A blood sugar two hours after breakfast on the third day of this regime determines further insulin readjustment. This method permits intensive hospital diabetic instruction and yet allows return of the patient to his gainful occupation in from four to seven days.

3. Since the formula here presented is entirely theoretical in its conception and derives its only merit from satisfactory and apparently safe clinical experience, the fact must never be overlooked that it should be modified to the need of the individual patient as determined by that patient's clinical response. It should not be used in the treatment of patients with myocardial damage.

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Metropolitan Building

SODIUM TAUROCHOLATE AND THE VIRULENCE OF HUMAN TUBERCLE BACILLI*

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DURING the course of studies attempting to disclose the factors accounting for the difference in gross colony characteristics of human and bovine tubercle bacilli noted on different culture mediums, particularly the striking difference on inspissated egg mediums (low spreading wrinkled form) and on potato mediums (elevated, heavy, limited form) as well as on inspissated whole blood mediums (thin veil-like spread growth[1, 2]), certain substances were incorporated in different nutrient mediums for the purpose of changing the physical-chemical properties of the medium, particularly to change the surface tension of the medium. Among those tried, one, sodium taurocholate in certain concentrations, produced decided changes in the nature of the resultant growth which led to a further study of the colonies and of the bacilli. Although our studies did not disclose the exact nature of the factors determining the characteristics of the gross growth on the various mediums, it did appear that by reducing the surface tension of these mediums to a common level there grew on these different mediums identical colonies of the so-called smooth-greasy type recorded as seen predominantly with virulent avian tubercle bacillus cultures on egg mediums.[3, 4]

The experiments were conducted with graded plantings from one milligram to one-millionth of a milligram and varying amounts of sodium taurocholate (Eastman Kodak Co.) were added to defibrinated cow blood, to egg white, to egg yolk, and to potato cylinder mediums which were then sterilized by inspissation or autoclaving as usual. All the mediums contained 3 per cent glycerol as is customary for growing human tubercle bacilli. With defibrinated cow blood, 1 per cent of sodium taurocholate inhibited the growth of small plantings; 2 per cent inhibited medium grade plantings; and 5 per cent inhibited moderately heavy plantings. With egg white, a poorer nutrient, the inhibition effects on growth were even more striking, and consequently the effect on the colonies was inappreciable. With inspissated egg yolk and potato cylinder mediums, inhibition was noted in the smaller plantings when the mediums contain 5 per cent sodium taurocholate but not when containing 2 per cent. Most striking, however, was the effect of the 5 per cent sodium taurocholate upon heavy plantings (one milligram or more per cubic centimeter seeding) in producing smooth, solitary, greasy appearing "avian-like" colonies on the egg yolk and potato cylinder mediums.

These colonies were, therefore, studied for their characteristics, regarding particularly the virulence of the bacilli and the permanency of the changes

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produced. The former (the virulence) was studied by graded inoculation of fine suspensions into guinea pigs, a quantitative method of virulence determination, and the latter (culture characteristics) was studied by transfer back

Fig. 1.—The effect of sodium taurocholate in egg yolk and potato cylinder mediums on the nature of the growth of human tubercle bacilli. The four tubes on the left are inspissated egg yolk (1 mg. per c.c. planting), the control being on the extreme left and in order the next three tubes contain 1, 2, and 5 per cent sodium taurocholate, respectively. The remaining tubes on the right (0.01 mg. per c.c. planting) are potato cylinder medium, the first of this set being the control and the last three consecutively containing 1, 2, and 5 per cent sodium taurocholate. The strain of virulent human tubercle bacilli pictured is strain "Gluckson" and the period of incubation seven weeks. Note the characteristic culture type of growth for egg and potato, and the change to the smooth, moist appearing globular colonies in the presence of 5 per cent sodium taurocholate.

and forth for several generations on both the inspissated egg yolk medium and the potato cylinder mediums with and without the sodium taurocholate (5 per cent).

A number of different strains of human tubercle bacilli were studied; most of them that had been cultivated on the laboratory mediums proved readily adapted to the culture tests, but a few recently isolated strains could not be grown on the 5 per cent sodium taurocholate mediums. The property of producing the solitary "so-called" smooth, greasy appearing colony was readily attained with both virulent and avirulent human tubercle bacilli on one transfer to egg or potato mediums, containing 5 per cent sodium taurocholate, and return to their original culture form was also attained by one transfer to the egg yolk or potato medium not containing the 5 per cent sodium taurocholate. This is well illustrated in the accompanying picture. The effect of the change wrought in the cultures by the sodium taurocholate on the virulence was studied by comparing the quantitative general virulence

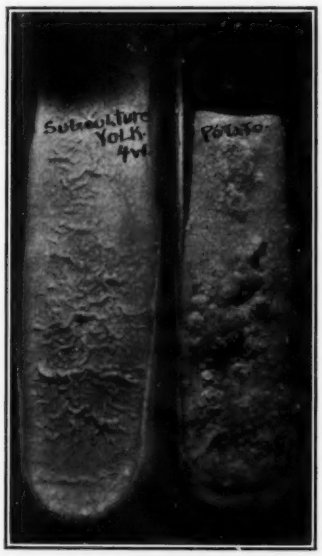

Fig. 2.—The immediate change to the characteristic growth for egg and potato after several transfers on egg or potato mediums containing 5 per cent sodium taurocholate when subcultured on egg yolk medium (left tube) or potato cylinder medium (right tube) without the sodium taurocholate. The strain of human tubercle bacilli is "Gluckson" and the incubation period four weeks.

of the taurocholate cultures with the ordinary cultures of the same strain, and using for this purpose both virulent and avirulent human tubercle bacilli.

The method of testing virulence, used for years in our laboratory for human and bovine bacilli, was based on the findings of one of us[5] from an extensive study of the effect upon the development of general tuberculosis in the guinea pig following various routes of infection. At that time it was found that the development of disease in the guinea pig differed little for practical purposes with the average strain of human or bovine bacilli, regardless of whether injected subcutaneously, intraperitoneally, or intravenously; and therefore, the subcutaneous method was chosen because of the ease of noting the development of disease by the enlargement of the local gland. The subcutaneous method has been used for general systemic virulence tests in guinea pigs and has proved of service in determining the virulence of human

and bovine tubercle bacilli with graded amounts of fine suspensions of these bacilli. The dilutions used are noted from Table 1 and duplicate tests (2 guinea pigs) were inoculated for each dilution being tested.

TABLE I

THE VIRULENCE OF A VIRULENT AND AN AVIRULENT STRAIN OF HUMAN TUBERCLE BACILLI AFTER GROWTH ON INSPISSATED EGG YOLK GLYCEROL-WATER MEDIUM AND INSPISSATED EGG YOLK GLYCEROL WATER 5 PER CENT SODIUM TAUROCHOLATE MEDIUM

STRAIN	MEDIUM GROWN ON	AMOUNTS OF SUSPENSIONS IN MG. INJECTED SUBCUTANEOUSLY IN GUINEA PIGS					
		10	1	0.01	10^{-4}	10^{-6}	10^{-8}
"Gluckson"*	Inspissated yolk Glycerol water			+†	+	+	0
"Gluckson"	Inspissated yolk Glycerol water 5% Sodium Taur.			+	+	+	+
"Human"*	Inspissated yolk Glycerol water	0	0	0			
"Human"‡	Inspissated yolk Glycerol water 5% Sodium Taur.	0	0	0			

*For a report on the Virulence of these two strains see: Corper, H. J., and Cohn, Maurice L.: The Viability and Virulence of Twelve Year Old Broth Cultures of Tubercle Bacilli Maintained at Incubator Temperature. Am. Rev. Tuberc. 28: 856, 1933.

†The result of examination of the guinea pigs at postmortem is recorded as + indicating a definite tuberculosis or 0 indicating the absence of macroscopic tuberculosis in any of the organs or tissues.

‡The bacilli used were taken from the third subculture on the indicated medium.

The results recorded in Table I indicate that although sodium taurocholate in 5 per cent concentration in inspissated egg yolk mediums (or potato cylinder medium, results not tabulated) causes an immediate change in the gross colony appearance to that of a smooth-greasy "avian-like" colony, but it does not enhance the virulence of avirulent human tubercle bacilli in spite of at least three continuous transfers on the taurocholate medium. A virulent strain of human tubercle bacilli would appear to have increased in virulence from the results obtained, but when analyzing these results they prove to be more apparent than real, in that a transfer back to a medium not containing taurocholate reveals the change to be rather one of temporary nature and not one of virulence increase. There are two factors which may contribute to this apparent change, one the increased concentration of the bacilli in the taurocholate colony, and the other the greater ease of preparing finer dispersed and more stable suspensions of the bacilli in the presence of the taurocholate.

In view of our findings with sodium taurocholate recorded above, and other observations not recorded here, we feel that the problem of correlation between rough colonies and avirulence, and smooth colonies and virulence for human and bovine tubercle bacilli merit further exhaustive investigation with careful control both as to virulence tests and the physical-chemical nature of the mediums. It would appear also that the characteristic of rough or smooth for human and bovine tubercle bacilli may represent changes dependent more or less upon the physical-chemical properties of the medium in close proximity to the individual colony.

SUMMARY

1. Virulent and avirulent human tubercle bacilli grown on an inspissated egg yolk medium or on a potato cylinder medium containing 5 per cent sodium taurocholate following heavy plantings produce smooth-greasy appearing "avian-like" colony growths which can be changed by a single transplant on the same mediums not containing taurocholate.

2. Avirulent human tubercle bacilli grown through several generations on egg or potato nutrient mediums containing 5 per cent sodium taurocholate do not show an increased virulence for guinea pigs in spite of the temporary physical change in the gross colonies.

3. Virulent human tubercle bacilli grown on egg or potato nutrient mediums containing 5 per cent sodium taurocholate show a transient apparent increase in virulence for guinea pigs.

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VII. AUTONOMIC IMBALANCE IN ANIMALS*

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DURING the last four years we have used several hundred animals in studying the qualitative and quantitative effects of drugs affecting the autonomic system (we use the term "autonomic system" to include the central and peripheral sympathetic and parasympathetic nerve fibers, as well as the individual metabolites affecting them). We were impressed with the striking variations in the reaction of animals to epinephrine, which is considered a typical sympatheticotropic stimulant, and the reaction following the administration of Desympatone† (active principle of Tissue Extract 568), which, as we have shown, stimulates the parasympathetic system.[2, 4, 5, 6, 7]

We were able to subdivide our animals (mostly dogs) into three groups or classes.

Group I.—Animals which are very susceptible to epinephrine, and relatively insensitive to desympatone.

Group II.—Those showing reverse phenomena, being particularly susceptible to desympatone and relatively insensitive to epinephrine.

Group III.—Animals showing rather a marked susceptibility to the effects of both types of drugs. In our experience we have not found a dog which failed to respond to the administration of therapeutic doses of both substances, although it is conceivable that such animals might be encountered.

This marked variation in susceptibility was emphatically called to our attention when, during one working day, tests were made upon two nonpregnant dogs weighing approximately 10 kilograms each, and anesthetized with morphine and chloretone. Fig. 1 shows the comparative effects of epinephrine and desympatone (Dog 301). This animal showed a slight reaction to desympatone, but was extremely sensitive to epinephrine. After administering 20 gamma ($^{20}/_{1000}$ milligram) of epinephrine, a marked rise in blood pressure was produced (Fig. 1, *A*). Simultaneous electrocardiograms showed dropped ventricular beats. This occurred at the height of the blood pressure rise. It is usually accepted that epinephrine causes an increase in the cardiac rate. This animal definitely showed a normal auricular rate, but the ventricular rate was cut down markedly on account of the ventricular asystoles (Fig. 2, *A*). In this tracing, reading from left to right, one sees an auricular complex marked *P.;* this is followed by QRS T-waves, which represent systole of the ventricles. The QRS complex is registered by very fine lines, requiring careful examination or the use of a magnifying glass for the untrained eye. Another normal P, QRS, T follows; then three P-waves representing three auricular systoles with

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†Different lots of tissue extract contain varying small amounts of histamine and choline compounds. Chemical studies are being made to remove them. The desirable clinical effects are attributed to "Desympatone," which is a distinct entity.

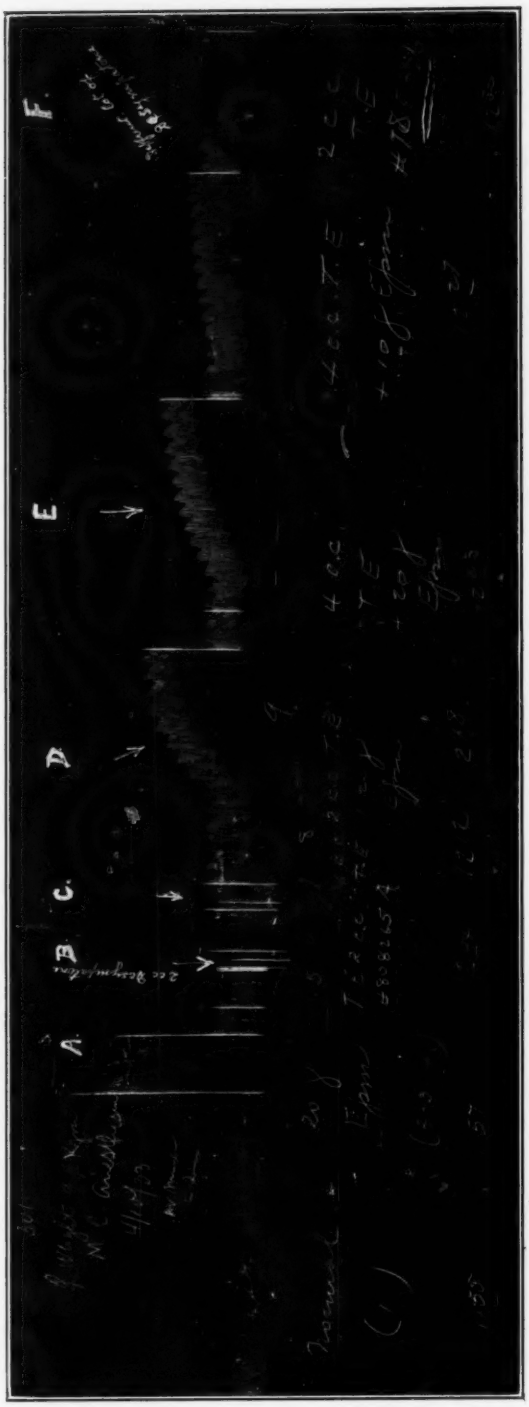

Fig. 1.—Showing effect of epinephrine and desympatone on Dog 301. This animal showed but slight reaction to desympatone, but was extremely sensitive to epinephrine.

only one ventricular response QRS, T; two normal cardiac cycles were again registered, followed once more by a ventricular standstill; this cardiac disturbance continued until it returned to its normal rhythm. The injection of

2 c.c. of tissue extract showed but a slight drop in blood pressure (Fig. 1, *B*); 4 c.c. of tissue extract also showed but a slight depressor effect (Fig. 1, *C*); 2 c.c. of tissue extract and 20 gamma of epinephrine brought about a marked rise in blood pressure (Fig. 1, *D*) and 4 c.c. of tissue extract still failed to

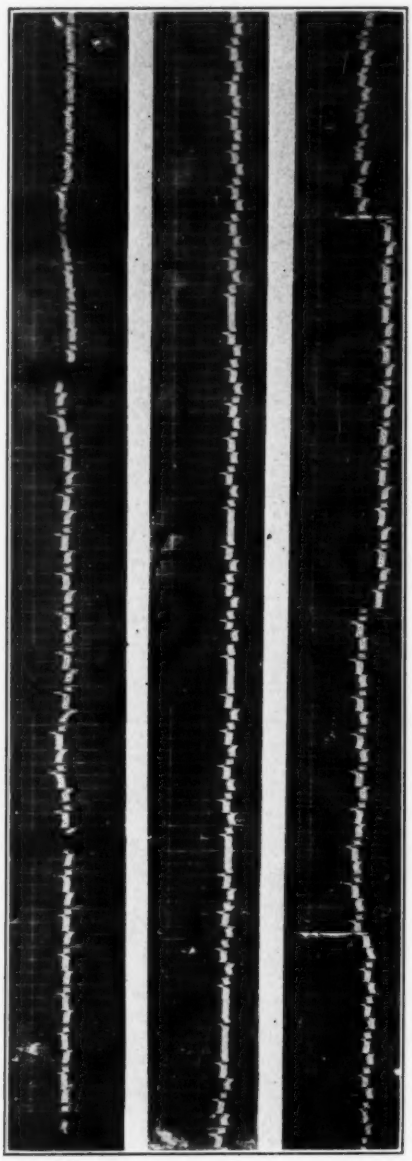

Fig. 2.—Normal electrocardiogram, Dog 301. *A*, Epinephrine produced numerous ventricular asystoles in this animal. *B* and *C*, Desympatone did not produce the characteristic effects upon the heart rate and T-waves in this animal. *D*, Desympatone administered with epinephrine did not influence the T-waves but prevented the ventricular asystoles seen in *A* when epinephrine alone was given. *E*, Desympatone from a different lot also failed to show the characteristic effects on this animal.

completely neutralize 10 gamma of epinephrine (Fig. 1, *E*). Taken after 2 and 4 c.c. of tissue extract respectively, the electrocardiograms (Fig. 2, *B* and *C*) did not show the characteristic effect upon the rate and T-waves. When the mixture of the two was administered (Fig. 2, *D*) tissue extract apparently prevented the blocking effect of epinephrine in this animal, but did not influence the T-wave. We have already shown in previous publications[4, 5, 6] that epineph-

rine and desympatone will affect the T-wave in opposite directions. In this animal the T-wave is not appreciably affected, since the T-wave in Lead 2 (Fig. 2), which we are continually registering, is very much like all the others seen in subsequent tracings.

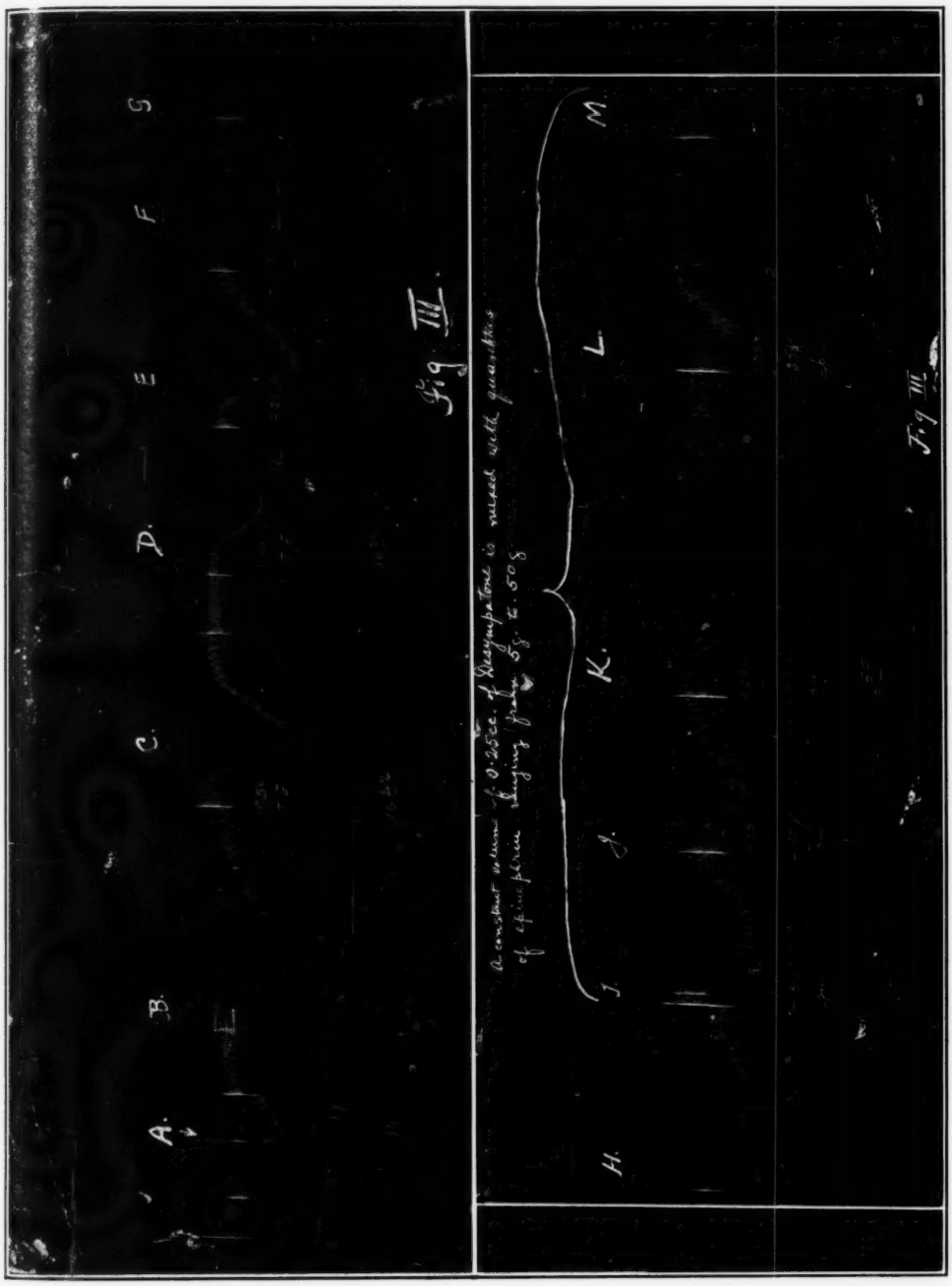

Fig. 3.

Thinking that possibly the tissue extract solution was weak, we used 2 c.c. from a different lot, but the effect upon the kymogram and electrocardiogram was about the same (Figs. 1, *F* and 2, *E*).

Figs. 3 and 4 show the blood pressure and electrocardiographic effects produced in Dog 302, following the administration of the *same* solutions. This animal proved to be very susceptible to desympatone and relatively resistant to epinephrine. Ten gamma of epinephrine caused a primary rise and secondary drop in the blood pressure (Fig. 3, *A*). The rate is uninfluenced. A slight

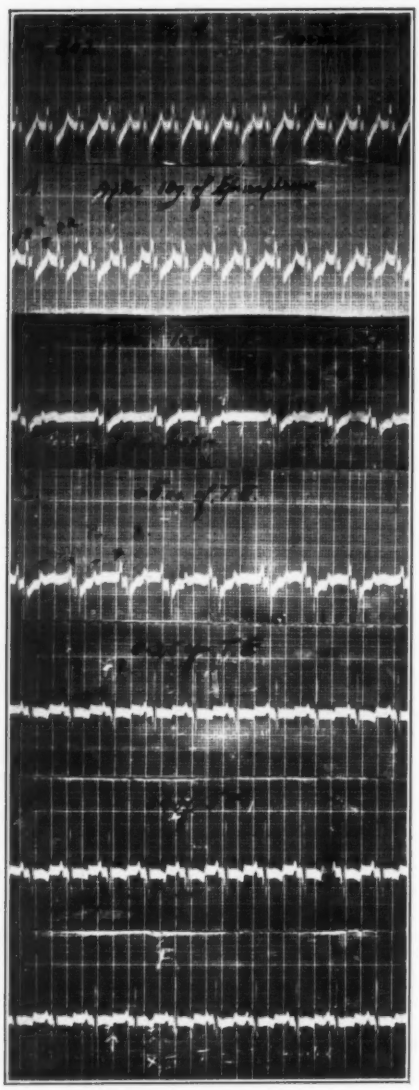

Fig. 4.

change developed in the character of the T-wave and in the R-T interval (Fig. 4, *A*). One cubic centimeter of tissue extract, employing the same lot used on the first animal, caused a marked depressor effect (Fig. 3, *E*). The electrocardiogram (Fig. 4, *B*) showed a definite sino-auricular bradycardia and sinus arrhythmia. The T-waves are markedly depressed as compared with the high T-wave seen on the normal tracing. The rate has been cut down from 144 to

84. Smaller amounts 0.5 c.c. (Figs. 3, *C* and 4, *C*) and 0.25 c.c. of tissue extract (Figs. 3, *D* and 4, *D*) showed proportionate drops and corresponding electrocardiographic changes. An injection of 0.25 c.c. of tissue extract (Figs. 3, *E* and 4, *D*) and 0.1 c.c. (Figs. 3, *F* and 4, *E*) again showed proportionate effects. As small a volume as 0.05 c.c. tissue extract (Fig. 3, *G*) showed a slight fall in blood pressure.

The method of standardization of tissue extract depends upon its neutralizing the effect of epinephrine upon the blood pressure of a dog. Fig. 3 (*I, J, K, L,* and *M*) shows the effects produced when a constant volume of 0.25 c.c. of tissue extract is mixed with quantities of epinephrine ranging from 5 gamma to 50 gamma. With 5 to 25 gamma of epinephrine the depressor action predominates in this dog. With 35 gamma (Fig. 3, *L*), the fall and rise are equivalent; and with 50 gamma of epinephrine, the pressor effect prevails (Fig. 3, *K*). Therefore, tested on this dog, 1 c.c. of tissue extract apparently corresponds to 120 gamma of epinephrine or 120 units, while if the previous dog was used as a test animal, 1 c.c. of the same solution would be equivalent only to 2.5 units, approximately 50 times less than in Dog 302. The very definite effect of repeated doses of desympatone on the T-wave should be noted. In the earlier records the T-wave was markedly upright (as shown by Fig. 4, *A* x *1*) and later on it became definitely diphasic (Fig. 4, *D* x *2*) and then inverted (Fig. 4, *F* x *3*). The P-wave in the normal tracing is extremely high (Fig. 4, *A Y 1*), much smaller in Fig. 4, *B Y 2*, and proportionately increases and decreases, depending upon the amount of desympatone administered. In these two animals the differences in the results are extremely striking; in the first animal (301) 4 c.c. of tissue extract failed to neutralize 10 gamma of epinephrine, while in Dog 302, 0.25 c.c. of the same solution completely neutralized 35 gamma of epinephrine. In an assay on Dog 301 alone this solution would appear to contain less than 2.5 units per cubic centimeter, while assays on Dog 302 would indicate the presence of 140 units per c.c. These extreme variations are seldom seen. We avoid such discrepancies by using a number of anesthetized atropinized animals in standardizing tissue extract. Marked variations in susceptibility of the components of the autonomic system have frequently produced apparent contradictions in the results obtained during our studies. We have learned to determine the relative susceptibility of test animals[7] in connection with research work on products of this type, as well as in such apparently simple studies as the U.S.P.X. bioassay of epinephrine solutions.[3]

The difference in tonus of the different components of the autonomic system should be kept in mind in studying drugs having a specific effect upon this system. Many of the contradictory results observed by different investigators may be due to these marked differences in the autonomic balance of the animals employed.[7] A subsequent paper will present methods of grouping animals according to their autonomic states.

At present it appears important to test out every animal first as to its reaction to epinephrine. As soon as the effect of epinephrine has worn off, the

response to desympatone should be recorded. These hormones are very quickly eliminated,[7] apparently without influencing the animal's response to other drugs.

CONCLUSION

In studying the quantitative effects of drugs acting upon the autonomic system, the susceptibility of the test animals should be determined relatively to standard solutions of epinephrine and desympatone (the active constituent of tissue extract). Such a procedure will eliminate many of the apparent discrepancies observed on unstandardized animals.

We wish to acknowledge, with thanks, the splendid assistance of Arnold Quici and Mabel E. Luccareni for their assistance in compiling the necessary data, and to Sharp and Dohme for supplies of Tissue Extract 568.

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INTRAVENOUS LETHAL DOSES OF AMYTAL IN THE DOG AND RABBIT AND A TABLE OF ANIMAL DOSAGES COMPILED FROM THE LITERATURE*

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RECENT barbiturate studies indicate that closer attention must be paid to various details and factors than has hitherto generally been done in establishing accurate dosages. Also in some cases results cannot well be interpreted because authors give incomplete information about what would appear to be essential points. Finally, comparisons are difficult owing to variations in criteria and toxicologic terminology.

Therefore, we wish (1) to present a summary of recent studies upon factors which need consideration, together with our own findings in regard to some of them, (2) to discuss the possibility of clarifying the toxicology terminology, (3) to present a mortality study in which we have tried to follow the discussed principles, and (4) to give a comparative dosage table in which, by our headlines, the topics are indicated about which we believe information is desirable for proper evaluation of the proposed values.

SUMMARY OF RECENT STUDIES

The Drug Used, Its Degree of Purity, the Form in Which Administered, the P_H.—"Crystalline sodium amytal has a lower M.L.D. than the prepared solution of amytal,"[29a] partly due to difficulties of avoiding slight decomposition and of changing all amytal into sodium amytal. Even pure sodium amytal crystals may contain not more than 0.1 per cent unchanged amytal.[2] Our dog experiments with intravenous, prepared sodium amytal do not give values that differ from those of Swanson and Shonle,[32] who used pure sodium amytal. Slight lowering of the intended P_H of 9.5 to 9.8 causes separation of traces of amytal with loss of hypnotic power.[32, 35]

Perfect *stability* of sodium amytal solutions of up to five months is claimed;[18, 21, 24] others note a tendency to deterioration on standing.[32, 35] Our pooled remnants after two months needed filtering and about 25 per cent increase in anesthetic dosage. Hence, for research work we use solutions on the day of preparation only.

A 10 per cent *concentration* is about 50 per cent more anesthetic in the white rat than a 2 per cent one, hypodermically administered.[21] Also, intravenous saline injections into rats revive those fatally poisoned with concen-

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trated solutions of sodium amytal.[1] On the other hand, rectal administration to dogs of 10 and 20 per cent solutions, respectively, give about similar results.[3]

As to *solvents*, ethyl glycol gives a more stable sodium amytal solution and dissolves amytal itself.[24] The pain factor necessitates rather dilute solutions for certain injections, and ethyl glycol must be studied further. A final acacia concentration of 10 per cent protects against circulatory effects (Ibid.).

In regard to *method of administration* and *rate of intravenous injection*, the rabbit shows an unusually large difference in lethal dosage between oral and other methods of administration.[10] Rabbits also survive operations better after oral than after subcutaneous administration.[23] And the lethal dose in this animal, and less so in the dog, declines very rapidly with increase in rapidity of intravenous administration.[32] Very rapid injection of but a few mils of even an animal's own blood may produce widespread disturbances, named "speed shock," with a possible early exitus.[12]

The total *number of animals*, and in mortality studies also the number in the fatal dose range, though not always available, would seem essential information for proper evaluation of any dosage. As to *sex*, adult female white rats are twice as susceptible to subcutaneous sodium amytal as are males, using deep anesthesia as a criterion,[21] but the two sexes are equally susceptible to nembutal.[1] Supposedly, the early *pregnancy* of 80 per cent of the amytal females is not changing the dose significantly. Rabbits do not show any sex difference when averaging many barbiturates after oral and intraperitoneal administrations;[10] the above nembutal report makes individual revision desirable. Very early *castration* makes the male need the male white rat dosage, whereas later castration reduces the dose needed;[1] however, the weekly production of anesthesia after early castration may have led to the development of some tolerance (q. v.). As to *age* and *size*, maturing male rats fall between adult males and females, and immature males and females give satisfactory results with female dosage.[21] In cats, 1.5 to 3.5 kg., the greater the weight, the slower the induction and the less the depth for the same dose per kilo, subcutaneously.[20] Our findings as to sex and size differences will be found under experimental data.

Regarding *external factors*, fluctuations in *room temperature* between 25° and 37° C. have not definitely influenced the M.L.D. of white rats in the fourteen series of experiments with intraperitoneal administration of 2 per cent solution of sodium amytal.[32] As to *excitement* and *fear* barbital-treated rats, caged with a rabbit, though not attacked by it, remain awake considerably longer than the controls.[13] As much as 75 per cent dose differences with the same lot of white rats is "probably due to differences in strains of rats, *seasonal* changes, etc."[26] With groups of only 20 to 30 animals and no sex separation, any seasonal variation has yet to be established. Pending this, a direct comparison of amytal dosage of January with barbital dosage of September[31] may be postponed. The possible influences of *diet*, *previous conditions of keeping animals*, and *barometric changes* are not reported upon. A *moist atmosphere* gives a more rapid recovery in monkeys treated with barbiturates than does a dry one.[11]

Tolerance of dog metabolism to amytal is not developed;[4] their example indicates intervals of about two weeks between administrations. Likewise, equally good anesthesia was obtained in fishes by repeating intraperitoneal injections up to five times, probably with ten-day intervals or more.[15] But repetition of injections within a few days of one another may necessitate twice the initial dose to produce anesthesia in the rat or death in the rabbit.[9, 21] Thus, probably, the immediately acquired tolerance is lost within a few weeks.

CLARIFICATION OF TOXICOLOGY TERMINOLOGY

Comparisons of doses are further complicated because the toxicology terms do not mean the same to every investigator. Thus, the common term "minimum lethal dose" has variable meanings.[33b] It may mean a dose just sufficient to kill an occasional animal; e.g., Nielsen et al.[22] (1925) give increasing barbital doses to six cats, have the one on the highest dose die, and call that dose the M.L.D. Is it really justifiable to label any dose the M.L.D. from such an orientation experiment? Likewise Page and Coryllos[24] (1926) inject increasing amytal doses intravenously into nine dogs with fatal outcome to the one upon the highest dose, 95 mg. per kg.; they further state: "In one dose approximately 95-100 mg. per kilogram is fatal." No dose was tried between 75 and 95 mg. And the dosage is misprinted in a subsequent paper in which reference to the above gives the M.L.D. as 110 mg. per kg.[31] (Their Table VI.)

To Maloney, Fitch and Tatum[19] (1931) the M.L.D. causes death in 50 per cent of the animals, whereas Knoefel, Herwick and Loevenhart[16] (1930) define it as the smallest dose which kills over 50 per cent of the cases.

According to Swanson[29] (1932, a), "the M.L.D. is determined by requiring not less than 60 per cent (of the rats) to die on the same dose." Shonle, Keltch and Swanson[26] (1930) write that the M.L.D. is that which causes death of all or a majority of the animals on that dose. In the amytal series of Coutière[3] (1932) it seems to be the dose just large enough to kill every animal on that dose.

Similar divergence holds for the term "average fatal dose."[7, 17, 32]

To eliminate some less useful new barbiturates one may divide 350 rats among 16 barbiturates to get a rough idea of the effective and fatal dosage,[22] and even in a few cases use a rat a second time with at least a two-week interval.[28] But though there be a distinct advantage in using the values from one comprehensive piece of work for comparative studies, it does not seem quite justifiable to employ the values of the just-mentioned investigators for detailed comparison among the more important barbiturates, including the effects of varying percentages of the so-called M.L.D.[30] The number of animals is too small, the concentration is variable and nothing is said about a constant sex ratio. Even the revision of this comparative dosage,[29b] though using more animals, fails to consider the sex question and to keep a constant concentration.

In view of these difficulties of nomenclature we agree with Trevan[33] (1927) that it would be preferable to abolish the term "Minimum Lethal Dose" and to substitute a term that would always mean the same. He proposes the term

median lethal dose to designate a dose which will kill 50 per cent of all animals in question in a large group picked at random, and suggests the abbreviation L D 50 for it. L D 100 would be an unequivocal abbreviation for the certainly fatal dose. Proofs are given that the L D 50 is "in the neighborhood of doses with the minimum relative error of determination." Regarding the number of animals required in such determinations, mathematical evidence is furnished to show that about 30 is near the optimum, that significant increase in accuracy requires a great increase in number, and that "diminution of the group below 30 leads to a very rapidly increasing error." Or, as Trevan puts it: "The ratio of the cost of the test to the accuracy obtained reaches a minimum in the neighborhood of this group number." However, with larger numbers of animals, one may determine several points along the straight part of the mortality curve, usually between about L D 20 and L D 80, by splitting up this number into smaller groups, and get results "which will have only a slightly greater error than that which is obtained by injecting the whole group with the average lethal dose." The total number required for the determination of these points depends partly upon the slope of the characteristic and partly upon the accuracy desired. Trevan gives examples of this and adds emphatically that all "discrepant" results must be included. He[13] further writes (1932, a): "The ideal way is to get a set of groups symmetrically distributed around 50 per cent mortality with doses separated by intervals such that the difference of the logarithms is constant, the doses themselves, therefore, rising by equal proportions and not equal arithmetical differences. The L D 50 can be arrived at by drawing on squared paper the best straight line through the points obtained and determining where it cuts the line for 50 per cent mortality."

As to establishing the minimum effective dose, except when actual surgical operation is the aim, criteria vary so greatly both for the same and for other species that comparisons of results of different authors become quite difficult. Could not the Council or some other authoritative body suggest one or two criteria for each species of animals by which first to establish the M.E.D. in each case of new hypnotics, the stream of which as yet seems far from being stemmed?

EXPERIMENTAL DATA

The solution of sodium amytal was prepared in the usual manner, adding 8.85 mil. of N/2 pure sodium hydroxide solution to each gram of amytal with proper stirring, avoiding excessive heating, and with subsequent dilution by freshly glass-distilled, sterile water so as to make the concentration 10 per cent of sodium amytal. The solutions were always filtered, kept between 24° and 28° C. during use, and employed within six hours. On three occasions the P_H was checked and found to be within the range of 9.5 to 9.8.

Our original plan called for completion of all experiments during the warm months of October and November, but an outbreak of plague interrupted our animal supply and made it necessary to continue into December and January; however, the estimated L D 50 for the 109 dogs of the warmer months was only one milligram higher than that of the 71 of the colder ones, and in the case

of the rabbits a similar difference was found; hence, no important source of error was introduced by the change of season. So as to have more uniform temperature conditions during the actual experiments, the room was warmed and the temperature kept between 21° and 27° C. and the animals were kept well covered during the amytal action.

The short-haired varieties of rabbits were bought in the open market, and kept for at least ten days on a constant diet of wheat, carrots, fresh greens, and water. A total of 120 rabbits was used, 60 males and 60 nonpregnant females. Food, but not water was removed twenty hours before giving amytal.

The 180 dogs were delivered from the city pound, all breeds and mixtures, 90 males and 90 nonpregnant females, and kept on a mixed diet; as much

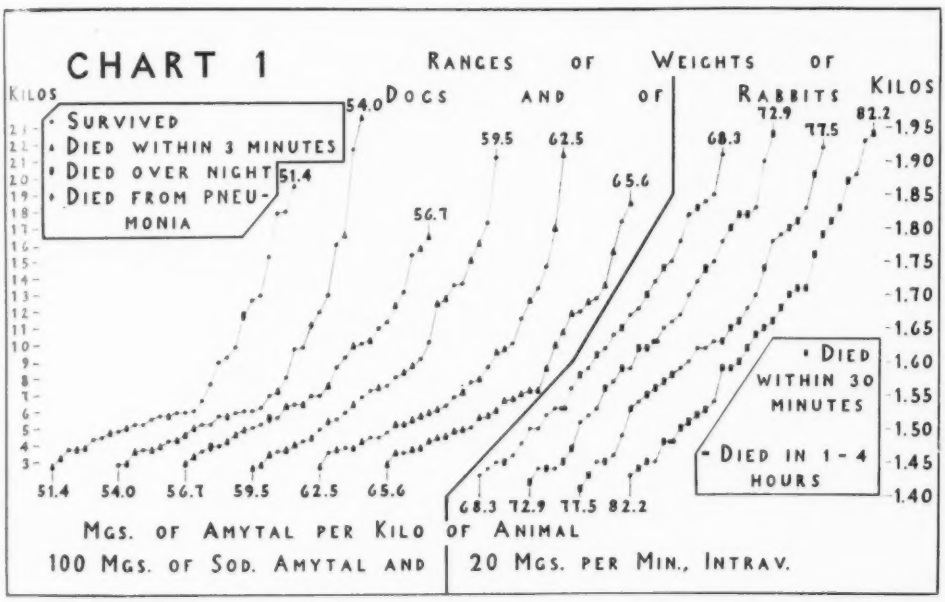

Chart 1.—Showing the weight ranges and uniformity of distribution of weights within each range; the size, dose, fate, method of administration and rate of injection for each and every animal (100 mg. per minute for dogs, 20 for rabbits); the increasing mortality with increasing doses, the very few delayed deaths and the higher mortality in the small dogs in the first four series.

bread and boiled meat as they would eat was given twenty-four hours before administering amytal; water was allowed all the time.

The determinations were completed in ten days upon the rabbits and in eleven days upon the dogs. On each day we tried to distribute the animals evenly among the doses.

The rabbit variation in weight was limited to the narrow range of between 1.40 and 1.95 kg. In the dogs, using only full-grown, apparently healthy animals, the weight range is that of the animals brought to us, with extremes of 2.7 and 23.4 kg. Chart 1 shows that the four series of 30 rabbits each, one for each dose, are very similar in their size distribution. In the six dog series the size distribution is sufficiently similar for biologic experiments.

Because rabbits are especially susceptible to very fast injections, we injected only 20 mg. of sodium amytal per minute into the marginal ear vein,

giving a minimum time of five minutes per rabbit. In case of the dogs the rate was 100 mg. per minute into the external saphenous vein. We are purposely giving our rate in terms of sodium amytal, so that later direct comparisons with pure sodium amytal mortalities may be more readily carried out.

In the rabbit the increase in dosage is 5 mg. of sodium amytal per kilogram for each new series. In the dogs, the dose increases in steps of 5 per cent (Logarithm 0.02119), so as to follow the latest suggestion by Trevan; this percentage increase seems to be very suitable for this type of medication. In either case the distribution around 50 per cent mortality was very satisfactory.

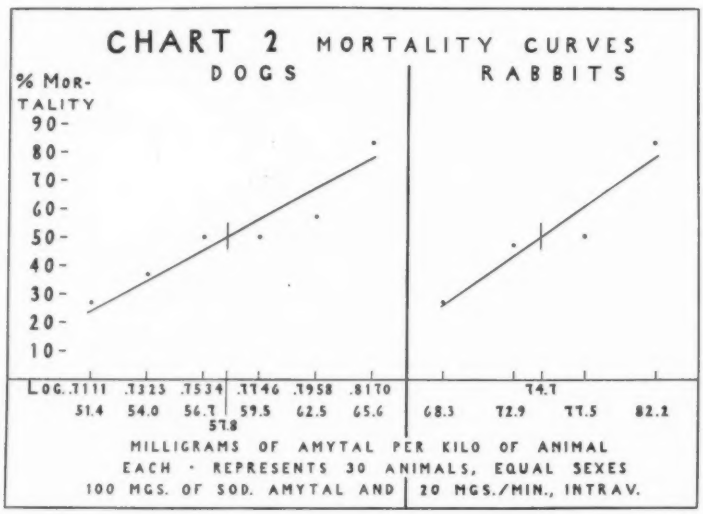

Chart 2.—Showing the percentage mortality of thirty animals on each of the indicated doses, the place where the best straight line fitting the points cuts the 50 per cent mortality line (L D 50) and the suitability of 5 per cent dose increases for intravenous amytal administration.

Our main results are shown in Charts 1 and 2 and in Tables I and II.

In view of the rather few mortality percentage points we have thought it best to draw the straight line that would best fit these, and thus to determine the L D 50 by seeing where this line would cut the 50 per cent mortality line. Because there seems to be a flattening in the middle values, one may try to use other types of curves; however, this will not appreciably alter the L D 50 value.

TABLE I

PERCENTAGE MORTALITIES IN RABBITS, 20 MG. OF SODIUM AMYTAL PER MINUTE

AMYTAL MG./KG.	15 MALES	15 FEMALES	TOTAL 30
68.3	27	27	27
72.9	73	20	47
77.5	33	67	50
82.2	87	80	83
All doses combined:	60 males 55 per cent mortality		
	60 females 48 per cent mortality		
	120 (total) 52 per cent mortality		

TABLE II

PERCENTAGE MORTALITIES IN DOGS, 100 MG. OF SODIUM AMYTAL PER MINUTE

AMYTAL MG./KG.	15 MALES	15 FEMALES	TOTAL 30
51.4	27	27	27
54.0	47	27	37
56.7	53	47	50
59.5	40	60	50
62.5	67	47	57
65.6	87	80	83
All doses combined:	90 males 53 per cent mortality		
	90 females 48 per cent mortality		
	180 (total) 51 per cent mortality		

To the nearest milligram of amytal itself, the L D 50 for the rabbits with our rate of 20 mg. of sodium amytal per minute, intravenously, is 75 mg. per kg., and for the dogs, using 100 mg. per minute, 58 mg. per kg. Comparisons with the rabbit values of Knoefel[16] et al. (1930; 68 mg.) and of Coutière[3] (1932; 65 mg.) are impracticable because they did not use a fixed rate of injection, and employed much fewer animals (see Table III); Swanson and Shonle's[32] (1931) pure sodium amytal value of 86 mg. of amytal is based upon one breed of rabbits, a faster rate and lower concentration, and Barron's[1] (1933) recent report makes it highly necessary to consider the concentration even with intravenous medication of amytal. In case of the dog our value checks very well with that of Swanson and Shonle[32] (1931) for pure sodium amytal; they report an M.L.D. (over 60 per cent fatality) of 63 mg. of amytal per kg.; 63 mg. from our curve gives an L D 67; concentration and injection rate were the same in both cases. A tentative value for the L D 25 in the rabbit would be 68, and for the dog 52 mg. of amytal per kg. Similarly, a tentative value for the L D 75 in the rabbit is 81, and in the dog 65 mg. of amytal per kg. with our injection rates. Thus, in either case, an increase of 13 mg. of amytal per kg. increases the fatalities from 25 per cent to 75 per cent.

In case of the rabbits, 59 out of 62 (or 95 per cent) died within thirty minutes, and 3 (or 5 per cent) died in from one to four hours after completion of injection (Chart 1). In case of the 91 dog fatalities, 88 (97 per cent) succumbed within three minutes of finishing the injection, whereas the remaining 3 (3 per cent) died during the night. In most cases the acute death in the dogs was immediately preceded by a short period of marked hyperpnea. Both acute and subacute deaths have been included in the mortality percentages as being directly attributable to amytal. One dog had evidently recovered from amytal after twenty-four hours; however, it developed pneumonia and died on the third day; this we have classed as an amytal survival. Similarly, another one of our survivals had pneumonia, but made a perfect recovery. The rabbits were kept for at least ten days after the experiment; none died during this time nor did any show signs of being ill. The dogs were killed at varying intervals of time (from one to eleven days) after recovery, to be used in a study of any possible pathologic changes (see later discussion).

In regard to any possible sex difference in the rabbit, Table I shows the extreme variations one may expect when using only 15 animals in one group

TABLE

AMYTAL DOS

ANIMALS AND INVESTIGATORS	NUMBER OF ANIMALS, FATAL RANGE IN ( )	SIZES IN GM. OR KG. SEXES	SUBSTANCE: PREPARED, OR PURE SOD. AMYTAL	PERCENTAGE CONCENTRATION
Fishes: (b)				
Keys and Wells	35 (15)	Wide range	Prepared	1
White Mice:				
Dox and Hjort			Prepared	1
White Rats:				
Nielsen, et al.	About 23		Prepared	4 and less
Swanson and Page	(39) 26	123-285	Prepared	10
Nicholas and Barron	250 pregn. and 45 nonpregn.	150-250 Females	Pure	10
	About 200	Over 200 Males	Pure	10
		Males	Pure	5
		Males	Pure	2.5
		Males	Pure	2.0
Swanson	55 (55)		Pure	5
Hoskins, et al.	33		Prepared	5
Drapkin, et al.		200	Prepared	(6)
Swanson and Shonle	936 (469)	About 60% Males	Pure	2
Shonle, et al.	18	100	Prepared	2
Fitch and Tatum	Over 40		Prepared	
Hoskins, et al.	24	Young	Prepared	5
Rabbits:				
Fitch and Tatum	Over 40		Prepared	
Page			Prepared	
Swanson and Page	94 (19)	1.4-2.3	Prepared	5
Page	8	1.3-2.7	Prepared	10
Shonle and Moment			Prepared	10
			Prepared	10
Dox and Hjort				
Fitch and Tatum	Over 40		Prepared	
Knoefel, et al.	16 (16)		Prepared	10
Swanson and Shonle	23 (23)		Pure	5
	15 (15)		Pure	5
	8 (8)		Pure	5
	10 (10)		Pure	5
	8 (8)		Pure	5
Coutière	29 (18)	1.8-2.5	Prepared	1.5-2
Holck and Kan'an	120 (120)	1.4-2.0 Equal sexes	Prepared	10
Cats:				
Swanson and Page	20 (20)	1.9-2.5	Solid Am.	
Eddy	10 (8)	Adults	Amytal suspension with acacia	Dilute
Mulinos	21	1.3-3.5 43% Males	Prepared	10
	33		Prepared	10
Koppanyi and Lieberson	15 (11)		Pure	10
Dogs:				
Swanson and Shonle	76 (31)	Adults	Pure	Solid (capsules)
Nielsen, et al.			Amytal	Solid (capsules)
Page			Prepared	10
Swanson and Shonle	89 (63)	Adults	Pure	Less than 10 and 20
Page			Prepared	10
Edwards and Page		Variable	Prepared	10
			Prepared	10

III

AGE TABLE

METHOD OF ADMINISTRATION AND MG. OF SOD. AMYTAL/MIN.	MIN. EFFECTIVE DOSE AS AMYTAL MG./KG. (a)	CRITERION AB = ABOLISHED R = REFLEX	LETHAL DOSES AS AMYTAL MG./KG.	CRITERION	REMARKS ON (1) STARVATION IN HOURS (2) DIET (3) ROOM TEMP. IN C° (4) SEASON (5) MISCELLANEOUS
Intrap.	40-54	Anesthesia (c)	50 60	Some die Uniformly fatal	(1) 24; (2) Constant
Intrap.	50-75	Anesthesia	200-210	Death during anesthesia	(1) 16; (2) Constant
Subcut.	57.5	Ab. Inner ear R.	140	Some die (?)	(1) 24; (2) Constant
Subcut.	40	Ab. Hypogastric R.	100	60% or more die	(4) January
Subcut.	91	Anesthesia			
Subcut.	182	Anesthesia			
Subcut.	255	Anesthesia			
Subcut.	272	Anesthesia			
Subcut.	275	Anesthesia			
Subcut.			209	Kills over 50%	
Subcut.	75	Anesthesia			(3) 31
Intrap.	50	Anesthesia	150	L D 30	
			200	L D 60	
Intrap.	71	Ab. Tail and inner ear R.	162	60% or more die	(1) 18-24; (3) 25-27; (4) Summer
Intrap.	72	Ab. Inner ear R.	180	All or majority die	(1) 24
Intrap.			115	L D 50	
Intrap.	50	Anesthesia			(3) 31
Oral			575	L D 50	
Oral	80-100	Anesthesia			(1) 48
Subcut.	45	Arousal impossible	110	60-100% die	
Subcut.	55-60	Anesthesia			
Subcut.	60-80	Anesthesia			(1) 48
Subcut.	30	Most sleep	150	L D 50	
Subcut.	40	Arousal impossible	170	"Toxic dose"	
Intrap.	50-75	Anesthesia			
Intrap.			90	L D 50	
Intrav.	45	Max. toleration	68	Kills just over 50%	
Intrav. 50			86	60% or more die	(5) Pure chinchilla
Intrav. 100			77	60% or more die	
Intrav. 200			63	60% or more die	
Intrav. 350			54	60% or more die	
Intrav. 500			50	60% or more die	
Intrav. variable	45-50	Anesthesia	65	L D 100 (?)	
Intrav. 20			75	L D 50	(1) 18-20; (2) Wheat, carrots, greens (3) 21-27 (4) October-January
Oral			110	60% die	(4) May
Oral			100	Large majority die	(1) 24; (2) Meat and crackers; (3) 20-25; (4) Summer
Subcut.	100 (Optimum)	Anesthesia			(1) 24; (2) Liver, heart and bread
Intrap.	70 (Opt.)	Anesthesia			
Intrav.			54	Majority (60%) die	(1) At least 12
Oral	63 (Min.)	Anesthesia	112	60% or more die	(2) Standard
Oral			250	First dose killing some	
Oral	100-125	Anesthesia			(1) 24
Rectal	100	Anesthesia	180	60% or more die	(2) Standard; (5) Prelim. enema
Subcut.	90-110	Anesthesia			(1) 24
Subcut.	70 (Opt.)	Anesthesia			
Intrap.	50	Anesthesia			

TABLE III

ANIMALS AND INVESTIGATORS	NUMBER OF ANIMALS, FATAL RANGE IN ( )	SIZES IN GM. OR KG. SEXES	SUBSTANCE: PREPARED, OR PURE SOD. AMYTAL	PERCENTAGE CONCENTRATION
Holck and Kanân	39	3-18 Adult Females	Pure (e)	10
			Pure	10
	26	3-20 Adult Males	Pure	10
			Pure	10
Swanson and Shonle	73 (29)		Pure	10
	27		Pure	10
	25		Pure	10
	15		Pure	10
	15		Pure	10
	15		Pure	10
Holck and Kanân	180 (180)	3-23 Adults Equal sexes	Prepared	10
Lower Primates				
Fulton and Keller	Several		Prepared	10
Weidman	2		Prepared	10

(a) All doses are in amytal as such. Pure sodium amytal has been multiplied by the factor given by Swanson, 0.902, to give amytal. All rates in intravenous injections are in milligrams of sodium amytal.

(b) The species were: Girella Nigricans[17]; Clinocottus Analis[11]; Apomatis Cyanellus and Caressius Auratus, a few of each.

(15 males or 15 females). The reversal of the high mortality for the two sexes in the two middle groups with good similarity in the lowest and highest dosage, indicates that there was no clear-cut sex difference. Similarly, in the case of the dogs, one cannot draw any definite conclusion from Table II. In view of the fact that in both cases the combined survivals on all doses somewhat favor the females (16 per cent more females survive in case of the rabbits and 11 per cent in case of the dogs), and because we have had to increase the sodium amytal dose by 5 mg. above the initial 60 per kg. more frequently in the females than in the males to produce surgical anesthesia (see Table III), it may be worth while to study this possible sex difference upon a large number of animals.

Although the fatalities do not with certainty show any sex difference, Tables IV and V show that on the whole the female rabbits do not have their body temperatures nor their respiratory rates quite as much depressed as do the males, and that they recover faster than the males. Although it is not very satisfactory to speak of a standard deviation for a few animals, our values also indicate that some males recover as fast as the females, but most do not. We have only included the rabbits of the warmer months, because with temperatures of about 21° C. not only are the initial body temperatures and especially respiratory rates less, but the temperature falls are more marked and complicated by shivering. The standard deviations also bring out the enormous variations between recoveries in rabbits who have been given the same dose and are lying side by side. Furthermore, actually the lowest respiratory rate came in our readings at fifteen, thirty, or forty-five minutes, so respiration rates begin to recover at a time when body temperatures are still falling (low point usually at second hour reading). Of course, most of the early workers with amytal reported depressed respiration rates and body temperatures, but as far as we know none reported upon any sex differences.

—CONT'D

METHOD OF ADMINISTRATION AND MG. OF SOD. AMYTAL/MIN.	MIN. EFFECTIVE DOSE AS AMYTAL MG./KG. (a)	CRITERION AB = ABOLISHED R = REFLEX	LETHAL DOSES AS AMYTAL MG./KG.	CRITERION	REMARKS ON (1) STARVATION IN HOURS (2) DIET (3) ROOM TEMP. IN C° (4) SEASON (5) MISCELLANEOUS
Intrap.	54	Anesthesia in 72%			(1) Mixed
Intrap.	59	Anesthesia in 90%			(2) 24
Intrap.	54	Anesthesia in 92%			(3) All year
Intrap.	59	Anesthesia in 96%			
Intrav. 100	36-45	Anesthesia	63	60% or more die	(4) May
Intrav. 200			68	60% or more die	(4) August
Intrav. 500			68	60% or more die	
Intrav. 750			63	60% or more die	
Intrav. 1500			59	60% or more die	
Intrav. 2000			54	60% or more die	
Intrav. 100			58	L D 50	(1) 24; (2) Mixed (3) 21-27 (4) Oct.-Jan.
Intrap.	50-70	Anesthesia			
Intrap.	55	Anesthesia			

(c) Anesthesia is used to designate complete, deep or surgical anesthesia or narcosis. Sedative and light sleep doses have been omitted.
(d) It seems probable but not certain that 10 per cent was used whenever no concentration is given for injected sodium amytal. This does not hold for oral administration in which probably extra water was given to the animal to rinse the stomach tube.
(e) Courtesy of the Eli Lilly Company.

As to the possible effect of variabilty in size, a careful comparison between the mortality percentage of the heavier 50 per cent (from 1.62 to 1.94 kg.) and the lighter 50 per cent (from 1.41 to 1.62 kg.), and, similarly, between the 10 heaviest and the 10 lightest within each of the four rabbit series, shows clearly that within the given limits of weights the size was not important in regard to fatality upon any given dose. This may also be seen by looking at the

TABLE IV

CHANGES IN RABBITS' BODY TEMPERATURES UNDER INTRAVENOUS FATAL RANGE DOSES OF SODIUM AMYTAL

ROOM TEMPERATURES 24.5 TO 27.0° C.

FEMALES	AVERAGE OF 7 (75 MG./KG.)		AVERAGE OF 9 (80 MG./KG.)		AVERAGE OF 4 (85 MG./KG.)	
	DEGREES C.	ST. DEVIATION	DEGREES C.	ST. DEVIATION	DEGREES C.	ST. DEVIATION
Normal	39.3	0.3	39.2	0.4	39.6	0.1
1 hour	37.3	0.6	37.1	0.7	37.3	0.3
2 hours	37.2	0.6	37.0	0.6	37.3	0.4
3 hours	38.2	0.8	37.7	0.8	38.2	0.5
4 hours	38.9	0.6	38.2	0.7	39.2	0.3
5 hours	39.2	0.5	38.8	0.5	39.3	0.2
6 hours	39.4	0.5	39.2	0.3	39.3	0.2

MALES	AVERAGE OF 8 (75 MG./KG.)		AVERAGE OF 3 (80 MG./KG.)		AVERAGE OF 10 (85 MG./KG.)	
	DEGREES C.	ST. DEVIATION	DEGREES C.	ST. DEVIATION	DEGREES C.	ST. DEVIATION
Normal	39.2	0.2	39.5	0.1	39.0	0.3
1 hour	36.9	0.4	36.6	0.4	36.5	0.8
2 hours	36.6	0.6	36.0	0.2	36.5	0.8
3 hours	36.7	0.8	35.9	0.1	36.9	1.1
4 hours	37.0	1.0	36.2	0.4	37.3	1.4
5 hours	37.8	1.2	37.2	1.4	37.9	1.4
6 hours	38.5	0.9	37.6	1.1	38.2	1.2

TABLE V

CHANGES IN RABBITS' RESPIRATION RATES UNDER INTRAVENOUS FATAL RANGE DOSES OF SODIUM AMYTAL

ROOM TEMPERATURES 24.5 TO 27.0° C.

FEMALES	AVERAGE OF 7 (75 MG./KG.)		AVERAGE OF 9 (80 MG./KG.)		AVERAGE OF 4 (85 MG./KG.)	
	RESP. RATES	ST. DEVIATION	RESP. RATES	ST. DEVIATION	RESP. RATES	ST. DEVIATION
Normal	228	35	236	12	224	7
1 hour	37	15	23	9	23	1
2 hours	51	24	26	8	38	20
3 hours	74	34	43	38	108	43
4 hours	134	65	78	55	194	33
5 hours	171	64	133	56	220	25
6 hours	219	35	193	40	259	22

MALES	AVERAGE OF 8 (75 MG./KG.)		AVERAGE OF 3 (80 MG./KG.)		AVERAGE OF 10 (85 MG./KG.)	
	RESP. RATES	ST. DEVIATION	RESP. RATES	ST. DEVIATION	RESP. RATES	ST. DEVIATION
Normal	221	21	235	16	237	26
1 hour	30	10	18	7	22	5
2 hours	32	7	24	6	32	15
3 hours	35	9	30	3	46	26
4 hours	49	15	35	11	68	49
5 hours	84	50	71	49	87	57
6 hours	115	61	93	57	115	67

distribution of fatalities along the total range of weights for each of the four rabbit doses (Chart 1). On the other hand, in case of the dogs there is a higher mortality in the weight range of from 2.7 to 5.4 kg. (66 dogs) in the lower four dosages as compared with the range of either from 5.5 to 8.9 kg. (55 dogs), or 9.0 to 23.5 kg. (59 dogs). The curve for the group of smallest dogs starts at a much higher level and is much less steep than either of the other two (actual mortality percentages for each of the six doses is 50, 58, 60, 60, 55, and 82, respectively, for this group). The main reason for this difference would seem to be the fact that in the smallest dogs the total dose would have been given in a very much shorter time than in case of a large one; thus, the very fastest total time for any one dog was one and six-tenths, and the very slowest was fourteen and six-tenths minutes, whereas in case of the rabbits the values are five and three-tenths and eight and eight-tenths minutes, respectively. It is also possible that the very small dogs may be more of the lap or house-pet type, and therefore be slightly less vigorous. The difference is not due to any very unequal sex distribution within this group of small dogs (19 males and 25 females in the four lower groups).

To get an approximate idea of what the L D 50 would be in the dog in the absence of extreme body weight variations, we have examined mortality curves for the middle two-thirds of each series (total of 120 dogs; approximate weight range of from 4 to 13.6 kg.), for the exact weight range of from 4.5 to 13.5 kg. in each series (117 dogs), and for the exact range of from 5.0 to 12.5 kg. within each series (96 dogs with not less than 15 in any of the six

groups). The L D 50 determined from each of these curves is 59 mg. of amytal per kg. of dog, or only 1 mg. higher than that for the total weight range.

Ravdin, Drabkin, and Bothe[25] (1931) report that repeated injections of good doses of sodium amytal into the white rat had no detrimental effect upon the heart, liver, lung, and kidney as judged by microscopic examination of these organs. In order to see whether or not lethal dose ranges of intravenously given sodium amytal injured the kidney or liver, sections were made from a large number of our dogs and stained with hemotoxylin and eosin. The slides from those that died acutely and those that were killed from one to eleven days after having been given the drug were then compared with the tissues of 10 apparently normal dogs which were killed by shooting. Table V shows that our street dogs, though apparently healthy, do show a good deal of pathologic changes.

TABLE VI

SUMMARY OF PATHOLOGIC CHANGES IN THE KIDNEY AND LIVER IN NORMAL AND IN AMYTAL-TREATED DOGS

	NUMBER OF	BOTH ORGANS NORMAL	SLIGHT CHANGES IN ONE OR BOTH	MORE SEVERE CHANGES IN ONE OR BOTH ORGANS
"Normal" dogs	10	50%	30%	20%
Acute amytal deaths	51	33%	50%	17%
Killed one to eleven days after amytal	55	33%	50%	17%

Dr. Homma reports: "The changes found in the liver were parenchymatous degeneration and fat infiltration and periportal round cell infiltration; in the kidneys, parenchymatous degeneration and interstitial round cell infiltration.

"All these changes, except the interstitial round cell infiltration in the kidney, which was found three times only among the dogs treated with amytal, were also found among the supposedly normal dogs. Also, no relationship between the changes and the dose of amytal given or the time after the administration could be established. So it must be concluded that the pathologic findings in the liver and kidney were not due to amytal."

SUMMARY

1. The introduction discusses the relative merits of the various factors which may modify amytal dosage and the desirability of clarifying the toxicology terminology by the adoption of the median lethal dose (L D 50) as a common criterion of fatality, and gives suggestions as to the manner of conducting mortality studies.

2. Ten per cent freshly prepared sodium amytal solution was injected intravenously at a rate of 20 mg. per minute of sodium amytal per kg. into each of 120 rabbits, equal sexes, from 1.4 to 2.0 kg., divided into four groups of thirty each, and using differences of 5 mg. of sodium amytal between the groups. From the best straight line that fits the four mortality percentage points, the L D 50 comes out to be 75 mg. of amytal itself per kg. of rabbit.

The value is not readily comparable with those of other reports, for others used lower concentrations and faster or variable rates of injection.

3. Similarly, the use of an intravenous rate of 100 mg. per kg. in 180 dogs, equal sexes, wide weight range (from 3 to 23 kg.), distributed among 6 groups of 30 dogs each, and with 5 per cent increases in dosage, gives an L D 50 of 58 mg. per kg. of amytal itself. This value agrees closely with the values reported by Swanson and Shonle[32] (1931) for a similar concentration and injection rate of pure sodium amytal.

4. Within the weight range of the rabbits, size played no rôle in the fatalities. In the dogs, the low range of from 2.7 to 5.4 kg. gave a higher mortality rate in each of the lower four dosages. However, by eliminating the extreme weights in various ways, the L D 50 in each case comes out 59 mg. per kg., or only 1 mg. higher than for the whole series.

5. No definite sex difference in regard to mortality is established, but a large scale study is suggested to test whether the female rabbit and dog may be a little more resistant than the male. In regard to body temperature and respiratory rate, the female rabbits were less depressed and on the whole recovered faster than the males.

6. No difference was found between mortalities in the warmer months of October and November as compared with the cooler months of December and January.

7. Microscopic examination of the liver and kidney with comparison of these tissues of supposedly normal dogs show that there is considerable pathologic change in our dogs and that no pathologic findings could be attributed to amytal.

8. Finally, a comparative dosage table for amytal has been compiled from the literature with addition of our own values and enough information added to make it possible for others better to judge the accuracy of each of the proposed dosages.

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THE VELOCITY FACTOR IN BLOOD TRANSFUSION

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SINCE the time of the Egyptian kings man has tried to alleviate the suffering of his fellow men by introducing the blood of a healthy individual into the body of one who is ill.[1] This therapeutic procedure has persisted throughout the ages and has passed periods of acclaim and disrepute. The rationale of its use and the technic of its administration have varied from time to time. Our knowledge of transfusions has accumulated during these years. Many investigations have been made and pertinent facts have been ascertained, but the problem of transfusion reactions remains unsolved.

In studying the responses to intravenous injections, Hirshfeld et al.[2] have described a definite clinical syndrome which they term *Speed Shock*. This is characterized by an acute onset following the injection of a substance intravenously and giving rise to salivation, vomiting, diarrhea, dyspnea, muscle atony or spasm coincident with changes in the blood and in the circulatory and respiratory systems resulting in a fall in blood pressure and respiratory distress. They were unable to elicit this syndrome when the injections were made slowly and point out the danger of giving intravenous medication rapidly, and suggest that posttransfusion reactions are due to a rapid injection of blood, especially where a syringe method is employed. They suggest that for clinical purposes a rate of 2 or 3 c.c. per minute should be the upper limit for amounts of over 100 c.c., and 1 c.c. per minute for smaller quantities.

We have studied transfusions from the clinical aspect, maintaining a standard technic and utilizing only whole blood. While we could not control all possible variables in the clinical procedure as well as one might do in a laboratory test, because the life of a patient always was involved, we have utilized all the ordinary methods available to the clinician to enable a proper interpretation of our results. We wished to determine if velocity was responsible for any of our transfusion reactions.

TECHNIC

Throughout this study we have used the Drummond Apparatus. This machine is essentially one of the stopcock type, designed to overcome the inconvenience and risk of error encountered in the use of apparatus employing a similar type valve (Fig. 1).

In this apparatus, as shown, it will be noted that the stopcock, which has three outlets, is mounted on the forward end of the machine. This moves in unison with the rotation of the barrel upon the plug, which is fixed. All air is

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displaced from the lumen by physiologic saline solution and during transfusion a constant hydrostatic saline pressure is maintained upon the syringe while the barrel is in the level position. The normal donor to recipient flow cannot be reversed because of mechanical locks, while at any stage of the transfusion saline may be drawn into the syringe and given to the recipient or the lock on the donor's side may be released, if the operator desires, and an infusion of saline given the donor. By no means are you able to aspirate the recipient's vein at any time. Volume is accurately recorded by a calibrated disc which turns one marking each time the 5 c.c. syringe is emptied into the recipient's vein. This apparatus enabled us to use whole blood without the

Fig. 1.

addition of citrate solution or any form of anticoagulant, and the ease of mechanical operation allowed us to give our entire attention to the reaction of the recipient.

All parts of the apparatus coming in contact with the blood were sterilized by boiling for twenty minutes. Other parts were immersed in alcohol or formalin solution for a like period. Our tubing was of an ordinary commercial type. We lubricated the steel piston with either sterile vaseline or a sterile citrated vaseline preparation and packed the open end of the syringe with the same material. This step in the preparation of the apparatus was demonstrated to us by Dr. John Noecker. It allows a thin film of grease to be spread over the inside of the glass syringe with each stroke of the piston and embodies the wax tube principle utilized by Kimpton Brown.[10] After

each transfusion we cleansed the apparatus with physiologic saline, hydrogen peroxide, and alcohol or ether.

We prefer that our donors always have a serologic examination, a typing, according to the Moss grouping,[1] and a cross-agglutination test with the recipient. We have found that the cross-agglutination test is never absolutely reliable unless observed for one hour. We know of no case in which we removed blood from a syphilitic donor.

A surgical technic was maintained in preparing the extremity for transfusion.[12] Tincture of iodine, 3½ per cent, and alcohol of 70 per cent strength were used on both anterior and posterior aspects of the area not covered by sterile drapes.

The pain incident to venipuncture was eliminated by a subcutaneous infiltration over the vessel to be tapped of a 1 per cent sterile novocaine solu-

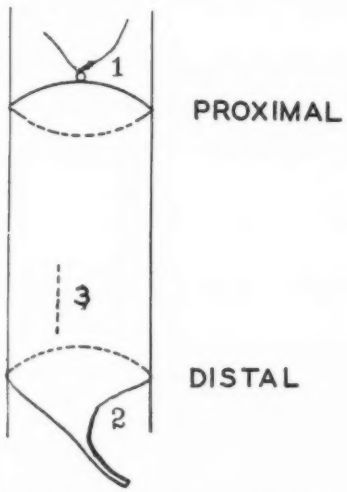

Fig. 2.—Showing vein held by suture material with single knot at 1 for retaining needle passed through incision at 3 and the blood current controlled by untied suture at 2.

tion. This procedure enabled the introduction of needles as large as 16 gauge without discomfort to either donor or recipient. When it was necessary to expose a vessel in order to introduce a needle into its lumen, we used novocaine anesthesia, making a small incision in the skin and carrying it down to the areolar tissue. Blunt dissection with a mosquito hemostat was used to isolate the vein, and it was brought to the skin surface by passing two short pieces of fine catgut under it. Separation of the ties, as shown in Fig. 2, enabled us to maintain complete control of the blood flow through the exposed section of the vessel. After the transfusion was completed and the retention ties removed from the vein it returned to its normal position. Temporary oozing was controlled by skin sutures and a pressure dressing.

Five minutes before giving the recipient the transfusion we recorded his blood pressure, temperature, pulse, and respiration, secured a complete blood count and a Schilling hemogram. Blood pressure was again recorded within five minutes after the transfusion and at the expiration of one hour, at which

time another blood count and Schilling hemogram were made. Urinalyses were made before and within twelve to twenty-four hours after transfusion. Temperature, pulse, and respiration were recorded at one, two, four, and six hours after transfusion. The following day another blood count and Schilling hemogram were secured.

The recipient's vein was tapped first. The needle being inserted in the direction of flow of the blood current. The efficiency of the venipuncture was tested by allowing at least 5 c.c. of the blood to flow into the syringe to which the needle was attached and immediately reinjecting this volume into the recipient's vein, his tourniquet having been released after the tap. We maintained the patency of the needle by injecting 10 to 15 c.c. of warm physiologic saline, during which time the tourniquet on the donor's arm was tightened and his vein tapped, the needle being inserted against the blood current. Connection with the apparatus was then completed, and with the appearance of blood in the syringe, from which it displaced physiologic saline, we considered the transfusion as started. Our estimation of total transfusion time includes from this instant until we withdrew the needle from the recipient's vessel. This was recorded by a stop watch.

The tolerance of the recipient to the blood was estimated clinically by giving the first 150 c.c. with extreme caution. We consider this a method of testing compatibility of bloods which should be regarded equally as important as the cross-agglutination test, providing the operator is trained in the clinical recognition of reactions. We always informed the patients of what was being done, when practicable, and asked them to tell us instantly of any new sensation or pain which they might experience. The facies, carotid pulsation and finger nails were observed for signs of cyanosis, distressed or altered expression, loss of pulse volume or acute circulatory collapse with resultant respiratory distress and embarrassment. These signs have been found clinically an earlier indicator of transfusion reaction than the substernal oppression, headache, and vomiting which all textbooks list.

Our estimation of velocity is based upon the total blood volume in cubic centimeters transfused, divided by the total transfusion time expressed in seconds. It is meant to accurately show the speed of transfer,[9] and is reported as cubic centimeters transfused per second. Velocity of transfusion depends upon the dexterity of the operator in temporarily connecting the donor's and recipient's veins. It is also influenced by the caliber of the needles used in the transfusion, it being impossible to propel in the same period of time, a given volume of blood through a needle of small caliber, as compared to one of larger lumen, providing we assume the propelling force to be the same. We have not attempted to approach the maximum in transfusion velocity but have maintained a standardized rate of transfusion which delivers the whole blood to the recipient in as near its natural condition as may be attained with present knowledge.

The danger of sudden death during transfusion is often totally absent from the operator's mind if the laboratory studies are favorable for transfusion. We have always kept prepared and ready for instant use a hypodermic of adrenalin and one of atropine sulphate.

DISCUSSION

These cases were assembled from the routine work in the wards of the Philadelphia General Hospital. Our cases were not selected nor have we given any consideration, in recording our findings, to the metabolic, toxic or morbid state of the individual patient. We have studied a group of 37 patients who were given 50 transfusions. Thirty-nine transfusions consisted in the transference of ordinary whole blood; ten were of the nonspecific-immuno type[4] and one was a citrated nonspecific-immuno transfusion.

Our interpretation of a posttransfusion reaction was based upon the study of a composite clinical picture in each case. We considered a reaction as having occurred if the patient showed a sudden change of facial expression; cyanosis; complained of acute pain; had a loss of vascular tone or respiratory distress; suffered a chill with an acute rise in temperature; displayed a marked decrease in the number of circulating R.B.C. per c.mm.; and an associated increase in the Schilling hemogram "multiple index"* (left shift) of 75 multiples; it having been shown by careful study that these changes in the clinical picture were not due to pathology present before the transfusion and unrecognized. Available material from our study leads us to believe that an increase in the multiple index of 75 constitutes the hemography of a posttransfusion reaction.

Kordenat and Smithies[11] found that in successful transfusions the posttransfusion leucocyte count was two or four times greater than before transfusion. Drinker and Brittingham[8] considered a rise in temperature of 2.5° F. as a reaction to the transfusion of citrated blood. We feel that transfusion reactions will be best interpreted when the decision is made, not upon a single clinical or laboratory factor, but is decided after a thorough study of the composite clinical picture of the individual case.

A total of 20,275 c.c. of whole blood was handled in this study, an average of 404 c.c. per transfusion. We considered the factor of acute cardiac dilatation only when the patient obviously had right heart failure. Dehydration is often present with the anemia, and before compatible blood could cause acute distention of a heart not already overburdened, the many venous lakes and the expansile factor resident in the venous side of the circulation would have to be taxed enormously. Crile has shown according to Col. C. Gordon Watson that the work of the heart increases in geometric ratio to the volume of blood, which in our transfusions was small. During the World War it was customary to transfuse large volumes of blood, usually 700 to 1,000 c.c. Major L. Bruce Robertson, C.A.M.C.[7] states that these cases never showed cardiac dilatation.

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*The "multiple index" indicates the number of times that a given shift is greater than normal. It is obtained by multiplying the Schilling index by 16. The normal Schilling index is 1/16. The normal "multiple index" is 1. The Schilling index is the ratio of shift cells (M, J, and St, the inefficient neutrophiles) to Sg or segmenters, the efficient neutrophiles. $\frac{M}{O} + \frac{J}{O} + \frac{St}{4} \cdot \frac{Sg}{64} = \frac{4}{64}$ or $\frac{1}{16}$. If the Schilling index of an ill individual is $\frac{3}{4}$, $\frac{M}{2} + \frac{J}{4} + \frac{St}{30} : \frac{Sg}{48} = \frac{36}{48} = \frac{3}{4}$, the "multiple index" is $\frac{3}{4} \times 16 = 12$. The Schilling index shows that there are 3 inefficient to every 4 efficient neutrophiles and the "multiple index" shows that the shift is 12 times greater than normal, that the Schilling index of $\frac{3}{4}$ contains twelve 16ths, or 12 multiples of the normal left shift. Crocker and Valentine: Hemography in Diagnosis, Prognosis, and Treatment, J. LAB. & CLIN. MED. 1934. (In press.)

Our studies showed that:

Average Velocity of transfusion of whole blood was 1.02 c.c. per second.

Blood Pressure.—

Five minutes after transfusion, 45 comparisons:

Systolic,	increased 30 times	average 12.1 mm. Hg
	decreased 13 times	average 14.0 mm. Hg
	unchanged 2 times	
Diastolic,	increased 24 times	average 13.2 mm. Hg
	decreased 14 times	average 9.6 mm. Hg
	unchanged 7 times	

One hour after transfusion, 38 comparisons:

Systolic,	increased 20 times	average 15.0 mm. Hg
	decreased 15 times	average 19.1 mm. Hg
	unchanged 3 times	
Diastolic,	increased 18 times	average 13.8 mm. Hg
	decreased 15 times	average 12.6 mm. Hg
	unchanged 5 times	

W.B.C. Count.—

One hour after transfusion, 50 comparisons:

increased 20 times	average 3,671 per c.mm.
decreased 30 times	average 3,205 per c.mm.

Twelve to twenty-four hours after transfusion, 31 comparisons:

increased 12 times	average 5,120.8 per c.mm.
decreased 19 times	average 6,551.5 per c.mm.

R.B.C. Count.—

One hour after transfusion, 50 comparisons:

increased 48 times	average 389,875 per c.mm.
decreased 2 times	average 712,500 per c.mm.

Twelve to twenty-four hours after transfusion, 31 comparisons:

increased 21 times	average 509,523.8 per c.mm.
decreased 10 times	average 429,500.0 per c.mm.

Temperature.—

Comparative readings at one, two, four, and six hours after transfusion, 38 comparisons:

increased 33 times	average 1.3° F.
decreased 5 times	average 2.8° F.

Pulse.—

Comparative readings at one, two, four, and six hours after transfusion, 38 comparisons:

increased 29 times	average $14\frac{12}{29}$ beats per minute
decreased 9 times	average $34\frac{2}{3}$ beats per minute

Urinalyses.—

Showed no changes of significance.

Schilling Hemogram.—

One hour after transfusion, 45 comparisons, of multiple index:

increased 27 times	average 17.1 multiples (left shift)
decreased 19 times	average 67.0 multiples (right shift)

Twelve to twenty-four hours after transfusion, 29 comparisons, of multiple index:

increased 12 times	average 37.8 multiples (left shift)
decreased 17 times	average 73.8 multiples (right shift)

Pathologic Report.—

Ultimate deaths	22	Autopsies	15

In no instance was death attributable to any other cause than the patient's disease. We have personally checked the histologic specimens of the kidneys in each of the fifteen autopsies and failed to find Bordley's post-transfusion kidney in any case.[6]

From an analysis of the composite clinical picture of posttransfusion reactions in our series of 50 transfusions, we found but two that showed evidence of a reaction. One was of a delayed type and one was of an acute nature. These two reactions represented 4 per cent of the total transfusions. The delayed reaction was due to the use of a so-called universal donor, without a cross-agglutination test having been made. The acute reaction, which conformed in every detail to Hirshfeld's description of *Speed Shock*, was found on recross-agglutination of the donor and recipient to be due to a definite incompatibility of the bloods which had either been missed at the previous examination or the titer of the agglutinating sera had been raised by the reaction.

Our observations to date include a series of over 125 transfusions of whole blood utilizing a similar technic. Two additional reactions have been noticed with the syndrome of *Speed Shock* in each case, but we have found that errors in compatibility were responsible for the reactions. There is a preponderance of opinion in the recent transfusion literature which emphasizes the fact that transfusion reactions are due to errors in compatibility (Parr and Krischner,[3] Polayes and Lederer,[2] Bordley,[6] Kordenat and Smithies,[11] also McClure and Dunn[13]).

While it has been held for years that the proper way to transfer blood was slowly, we have found that when the blood is compatible and untreated by extraneous chemicals, it may be given to the recipient according to our technic, at an average velocity of 1 c.c. per second without becoming a factor in the clinical picture of a posttransfusion reaction. We feel that keeping the blood out of contact with the vascular endothelium, which is its natural environment, for a prolonged period of time is undesirable.

CONCLUSIONS

1. The Drummond apparatus for blood transfusion is dependable and the mechanical principles which it embodies facilitate the transfusion of whole blood.

2. Anesthesia with a 1 per cent novocaine solution, as used in this study, eliminates the pain incident to venipuncture and should have a wider use in transfusion work.

3. The Schilling hemogram may be utilized in the study of transfusion reactions.

4. The *Speed Shock* syndrome was not elicited in the transfusion of whole blood at an average velocity of 1 c.c. per second except in cases where an incompatibility was demonstrated.

5. The study of a composite clinical picture is a preferable method of interpreting posttransfusion reactions.

6. Further studies on the velocity of transfusion of compatible whole blood are desirable.

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THE VALUE OF THE MONKEY FOR THE STUDY OF THE LAXATIVE ACTIVITY OF PHENOLPHTHALEIN, ESPECIALLY IN COMPARING DIFFERENT SAMPLES OF THE DRUG*

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MILLIONS of doses of phenolphthalein are taken annually in the United States for the well-known laxative action which it produces. For this purpose two general types of phenolphthalein, white and yellow (the more impure form), are placed on the market by manufacturers. The yellow product induces a much more powerful action than the white. Also, there is a considerable variation in the activity of samples of phenolphthalein from different manufacturers. Up to the present time the laxative activity of a particular sample could be estimated only by the use of human subjects, under inconstant conditions. A method of assaying this activity by the use of the monkey has been developed in the work described here.

All reports of the action of phenolphthalein in the lower animals fail to describe a laxative or purgative action similar to that in man except after relatively huge doses, usually requiring repetition. Such effects have been recorded by Abel and Rowntree[1] in dogs, van der Willigen[2] in cats, and Fleig[3] in dogs and rabbits. Vámossy[4] obtained no laxative action in dogs after a dose of 5 gm. and Führer[5] found the drug completely inactive in mice after 20 mg. were given. On the other hand Loewe and Lange[6] reported a softening of the

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feces of mice after moderate doses (17 mg. per kilogram body weight), but failed to characterize their observation with a convincing description.

Fleig[3] expressed the situation admirably in the following statement: "It is to be regretted that an action, so intense in the case of man, hardly develops at all in the case of the animal and consequently cannot be studied with all the scientific precision of experimental analysis. Perhaps experiments performed on the monkey would be more fruitful; it would be interesting to try this animal." During the twenty-five years following this recommendation, no record of any one's having tried it has come to our attention.

EXPERIMENTAL

The samples of white and yellow phenolphthalein used in the experiments recorded here were made by the same manufacturer. The white was of U.S.P. grade and the yellow fulfilled all U.S.P. requirements except those concerning color. The latter possessed a very light canary yellow color in the powdered dry state, but in alcoholic solution showed a brownish yellow color modified by a slight fluorescence.

A series of 14 young white rats, weighing from 35 to 190 gm. each, received doses up to as much as 600 mg. (weight of rat, 135 gm.) of white and 84 mg. (weight of rat, 105 gm.) of yellow. Of the more than 500 doses given, only 2 (100 and 300 mg. of white phenolphthalein, respectively) were followed by a demonstrable softening of the feces. In both cases the rats failed to respond to a second and larger dose, furnishing further evidence of the inconstancy of the laxative action of phenolphthalein in the lower mammals.

A series of 5 cats, weighing from 2.2 to 4.0 kg., were given massive (5 to 10 gm.) doses of both white and yellow phenolphthalein in suspension in water or milk by stomach tube. Only repeated doses proved effective.

A dog, weighing 18 kg., showed no laxative action after a dose of 10 gm. of yellow phenolphthalein.

At this point we decided to try monkeys, although Fleig's suggestion had escaped our attention. These animals (Macacus rhesus), weighing from 2.0 to 3.5 kg., were found to be most suitable for the study of the drug. They were maintained on a regular diet of milk and eggs twice daily and fruits and vegetables once a day. Bone meal, cod liver oil, and at times blood meal were mixed into their fruit meal.

Each dose of phenolphthalein was accurately weighed on an analytical balance and then was quantitatively deposited in the interior of a piece of banana and sealed in. On feeding the piece of banana the monkey was closely watched to ascertain whether he consumed the material entirely. More pieces of banana then were fed to insure complete ingestion of the dose. If the quantity was sufficient to produce laxative action the effect would be evident in any case within from three to twenty-two hours, but usually within from six to twelve hours. After such a dose the stools became formless and semifluid, or if the dose was much above the minimum they became actually fluid. The former type of action properly is termed laxative, the latter purgative. When a minimum active dose was given, the action was always laxative and consisted of only a single action or of 2 or 3 at the most.

In order to establish the minimum therapeutic dose, gradually increasing quantities of the drug were given, with intervals of 1 mg. for doses less than 10 mg. and of 2 mg. for larger doses. Between each successive trial at least three full days were allowed to intervene. Finally that level was reached, below which no action occurred and above which laxative action always took place. This point has been determined for white phenolphthalein in 9 monkeys and for yellow in 5, as shown in Table I.

TABLE I

MINIMUM THERAPEUTIC DOSES OF WHITE AND YELLOW PHENOLPHTHALEIN FOR MONKEYS

MONKEY	WHITE PHENOLPHTHALEIN	YELLOW PHENOLPHTHALEIN	RATIO: $\frac{\text{WHITE}}{\text{YELLOW}}$
	mg. per kg. body weight	mg. per kg. body weight	
1	2.8	0.8	
1		0.7	3.7
2	6.8	3.0	2.3
3	4.8		
4	5.0	0.9	
4		1.3	4.5
7	4.0		
8	4.1	1.5	2.7
9	2.1		
9	2.1		
10	5.9		
10	5.7		
11	4.1	0.6	6.8
Average	4.4	1.4	3.3

It will be noticed that the dose for 4 monkeys was redetermined with practically no variation from the level first established. The minimum dose for different animals, however, varied quite widely, as does the dose for human subjects. In one instance (Monkey 11) there is even a wide deviation from the average ratio of the dose of white phenolphthalein to that of yellow. As is usual in the case of experiments with animals, therefore, dependence should be placed rather in results obtained with several subjects than in those from a single animal.

The average doses thus obtained show the sample of yellow phenolphthalein to be about 3 times as powerful in laxative action as the white. This figure corresponds closely with the relative effect of these samples in human subjects. Other samples of phenolphthalein also have been assayed by using these same monkeys, and the results again confirmed the experience obtained with human subjects.

The field remains open for physiologic studies of the action of phenolphthalein on monkeys, with results which may enlighten us further as to its action on man.

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THE FUNGICIDAL POWER OF PHENOL DERIVATIVES*

I. Effect of Introducing Alkyl Groups and Halogens

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SIGNIFICANT of the unsatisfactory status of the present therapy of tinea infection is the large number of remedies used in its treatment. Considering the increasing incidence of the disease, its marked tendency to recur, and the frequency with which it persists in spite of prolonged treatment, comparatively few investigators have interested themselves in this particular problem of therapy. Apparently the first to do so were Bokorny[1] in Germany and Schamberg and Kolmer[2] in this country. Others whose publications have appeared at intervals in this field are Myers and Thienes,[3] Kingery and Adkisson,[4] Osborne and Hitchcock,[5] Schamberg, Brown and Harkins,[6] Bonar and Dreyer,[7] Smyth and Smyth,[8] and Legge, Bonar and Templeton.[9] These studies indicated that such phenol derivatives as thymol and salicylic acid which have long been known for their clinical usefulness have a just claim to their popularity. They also indicated that there might be other phenol derivatives less irritating than thymol but more effective toward fungi.

In recent years numerous studies of the *germicidal* properties of alkyl phenol derivatives have been made. Included in these is the work of Laubenheimer,[10] Johnson, Hodge and Lane,[11] Schaffer and Tilley,[12] Klarmann and his collaborators,[13] Rettger, Plastridge and Valley,[14] Coulthard, Marshall and Pyman,[15] Read and Miller[16] and others.[17, 18, 19, 20, 21] Substitution of halogens in phenols has been studied by Koch,[22] Bechold and Ehrlich,[23] and Klarmann and Von Wowern.[24]

The present work was done in an attempt to follow up the work of Kingery and Adkisson[4] and find, if possible, derivatives of phenol which might be used internally as therapeutic agents. As a first step it was thought desirable to learn the effects on fungicidal properties produced by the introduction of various types of groups into phenol.

Test Organisms.—The entire group of cultures used in this work was obtained through the kindness of Dr. Fred Weidman of the University of Penn-

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sylvania Medical School. The group was composed of the following fourteen yeasts and molds, practically all of which are known to be of a pathogenic nature—*Monilia tropicalis*, Sporotrichum, Cephalosporium, *Microsporon fulvum*, *Trichophyton interdigitale*, Blastomyces, *Monilia candida*, *Monilia pinoyi*, *Monilia metalondinensis*, *Monilia macedoniensis*, *Penicillium brevicaule*, *Cryptococcus histolyticus*, *Coccidioides immitis*, *Trichophyton purpureum*.

Compounds Tested.—Thirty-seven derivatives of phenol were tested with regard to their fungicidal power. Phenol, itself, was used as a basis for comparison. We are indebted to Dr. E. Klarmann of the Plaut Research Laboratory of Lehn and Fink for his kindness in furnishing samples of the four ethers of resorcinol listed in the table. A few of the compounds were prepared in this laboratory. Included in this group were 3, 5-diethyl phenol; 3, 5-dibutyl phenol, n-amyl salicylate; and 2, 4-dinitrophenol. The m-cresol and p-chlorophenol were purified before use. For purposes of comparison two other common fungicides, iodine and mercuric chloride, were also tested.

EXPERIMENTAL

Method of Testing.—The method used by Kingery and Adkisson[4] in their fungicidal tests has been followed with certain minor changes. Sabouraud's dextrose agar was used for the solid media. This was prepared by adding 10 gm. "Difco"-Bacto peptone, 40 gm. crude glucose, and 15 gm. agar to 1,000 c.c. tap water. After the media had been adjusted to a pH of 5.2 to 5.4, it was autoclaved for twenty minutes at fifteen pounds pressure. The liquid medium used was dextrose broth adjusted and prepared in the same manner with the omission of the agar. Stock cultures were grown on the solid media.

Culture tubes containing from 10 to 15 c.c. of broth were planted with a loopful of yeast from the stock tubes, thoroughly mixed and grown, seventy-two hours at 37.5° C. in case of the yeasts and longer at room temperature in case of the molds.

The compound, the fungicidal power of which was to be determined, was dissolved in water whenever possible and diluted to the desired concentration. In a few cases it was necessary to use dilute solutions of alcohol, glycerol, sodium carbonate or potassium hydroxide as solvents.

To 2 c.c. of the drug solution contained in a sterile test tube was added 1 c.c. of the broth suspension of the yeast or mold.

The mixture was then shaken and at the end of one minute, thirty minutes, and sixty minutes several loopfuls were transferred to separate sections of a plate of Sabouraud's dextrose agar. The plates had previously been divided into quarters by marking on the bottom of the Petri dishes with a wax pencil. The fourth quarter was used as a control. Transplants were made to this section from a tube containing 2 c.c. of sterile distilled water and 1 c.c. of yeast suspension.

The plate was then incubated for a period of time depending upon the organism being tested. The yeasts required seventy-two hours at 37.5° C. while it was found that the molds gave the best results when left at room temperature at least a week. All plates were left at room temperature for three weeks when final readings were taken.

All tests were made at room temperature which varied from 19° C. to 23° C.

Choice of Three Organisms.—Because of the large amount of work involved in the preparation of cultures it was found necessary to limit the number of organisms used.

Preliminary tests made with the entire group showed that three organisms, *Monilia tropicalis,* Sporotrichum, and Cephalosporium, were the most representative. *Monilia tropicalis* has the appearance of a typical yeast and shows vigorous growth in the incubator. Sporotrichum seems to lie between the yeasts and molds in form while Cephalosporium is more nearly a true mold. The latter two organisms grow quite vigorously at room temperature. All three show more than average resistance to the fungicidal agents tested. All compounds were tested against *M. tropicalis* which showed the highest resistance in all cases, while Sporotrichum and Cephalosporium were used as checks with all of the more effective compounds but not with the simpler phenols.

Control of Environmental Factors.—Temperature: Since in the case of bactericidal studies an increase in temperature has been reported to cause a marked increase in the germicidal strength of various drugs, a few tests were carried out to determine to what extent the working temperature should be controlled for satisfactory results in fungicidal studies.

An increase in temperature from 10° to 30° C. was found to increase the fungicidal ability of the drug slightly. However, the difference between the effect at 21° and that at 30° C. was small enough to make it seem unnecessary to take any special precautions to control the room temperature while making the tests.

Solubility.—The most troublesome factor which had to be contended with throughout the work was the near insolubility of a large number of the compounds. An effort was made in every case to obtain an aqueous solution of the drug to eliminate any possible fungicidal action of the solvent. However, it was found necessary in a few cases to use 10 per cent alcohol, 1 per cent sodium carbonate, 30 per cent glycerol, and 0.5 per cent potassium hydroxide solutions as solvents. None of these solvents themselves were found to kill the organisms under the conditions of our experiments.

It has been suggested that the insolubility of compounds may be closely connected with their ability as antiparisitic agents.[20] When a drug which is highly insoluble in water comes into contact with the lipoids of the cells in which it is more soluble, a rapid absorption of the drug by the cell is thought to result. All of the compounds which have been tested in this work and found to be highly toxic to yeast and molds are very slightly soluble in water. It was found that a water soluble, colloidal trade preparation of chlorothymol known as "clymocol" was not as effective a fungicidal agent as the crystalline chlorothymol.

While using 1 per cent sodium carbonate solution as a solvent, it was found that the fungicidal power of a compound dissolved in the carbonate solution was less than that of the same compound in an aqueous solution. Salicylic acid dissolved in water at a dilution of 1-1500 is more effective as

a fungicide than salicylic acid dissolved in 1 per cent sodium carbonate at a dilution of 1-500 or sodium salicylate dissolved in a 1-500 aqueous solution. On the other hand, thymol, carvacrol and chlorothymol are only slightly less effective in a sodium carbonate solution. Compounds with stronger acid properties are apparently more affected by being used in an alkaline medium than more weakly acidic substances.

Hydrogen Ion Concentration.—By use of a quinhydrone electrode the pH of the dextrose broth was found to be 5.3. The 1 per cent Na_2CO_3 solution had a pH of about 9 as did also a mixture of 1 c.c. of broth and 2 c.c. of 1 per cent Na_2CO_3 solution.

It has been indicated before that the sodium carbonate solution did not prevent the subsequent growth of the yeast and molds. It is evident, therefore, that a marked shift in the hydrogen ion concentration toward the basic end of the scale does not produce a fungicidal action.

Results of Fungicidal Tests.—Table I summarizes the results of the fungicidal tests made on the three organisms selected. The maximum dilution of each compound effective at thirty minutes and the phenol coefficient calculated by using the thirty-minute tests as a basis are given for each organism. The phenol coefficients were calculated by dividing the maximum effective dilution of a compound at thirty minutes by the maximum effective dilution of phenol at thirty minutes when using the same organism. Where not enough tests were made with a compound to make possible the determination of a definite coefficient, an approximate coefficient is indicated.

DISCUSSION

It may be seen from the table that certain structural features are necessary in a compound for it to possess high fungicidal power. The substitution of one or more alkyl groups on the benzene ring in phenols increases the fungicidal power, the resulting phenol coefficient depending upon the number of carbon atoms in the side chain. Compounds containing an alkyl group with six carbon atoms arranged in a straight chain have the highest coefficients as far as it is possible to tell from present results. The position in the ring occupied by the substituted alkyl groups makes no striking difference although the work has shown that the meta and para compounds are slightly stronger in fungicidal power.

Dialkyl compounds are stronger in fungicidal power than the corresponding monoalkyl compounds. One would anticipate that a dihexyl phenol would have a very high fungicidal ability except that its use is probably prevented by its insolubility. In the case of the dihydric phenols it has been shown that it matters not whether the alkyl group is substituted for a hydrogen atom of the benzene ring or a hydrogen atom of the hydroxyl group. The isomeric compounds, n-hexyl resorcinol and the n-hexyl ether of resorcinol, have coefficients which are nearly identical.

The indications are, from the five compounds studied, that the introduction of one or more nitro groups into phenols does not increase the fungicidal power materially.

TABLE I

FUNGICIDAL TESTS ON SUBSTITUTED PHENOLS

(Time: Thirty Minutes)

COMPOUNDS	*M. Tropicalis*		*Cephalosporium*		*Sporotrichum*	
	EFFECT. DILN.	PHENOL COEFF.	EFFECT. DILN.	PHENOL COEFF.	EFFECT. DILN.	PHENOL COEFF.
1. Phenol	1-75	1.0	1-110	1.0	1-150	1.0
2. o-Cresol	1-175	2.3	------	-----	------	-----
3. p-Cresol	1-190	2.5	------	-----	------	-----
4. m-Cresol	1-200	2.6	------	-----	------	-----
5. 4-OH-1, 2 Dimethyl benzene	1-500	6.6	------	-----	------	-----
6. 4-OH-1, 3 Dimethyl benzene	1-475	6.3	------	-----	------	-----
7. 2-OH-1, 3 Dimethyl benzene	1-400	5.3	------	-----	------	-----
8. 2-OH-1, 4 Dimethyl benzene	1-350	4.7	------	-----	------	-----
9. 3, 5-Diethyl Phenol (0.5% KOH)	1-2,000	26.6	1-2,000	18.2	1-2,500	16.6
10. 3, 5-Dibutyl Phenol (1% Na_2CO_3)	1-±11,000	±160.0	------	-----	------	-----
11. Thymol	1-2,000	26.6	1-3,000	27.3	1-3,000	20.0
12. Carvacrol	1-2,000	26.6	1-2,500	22.7	1-3,000	20.0
13. o-Nitrophenol	1-±500	±6.6	------	-----	------	-----
14. m-Nitrophenol	1-±400	±5.3	------	-----	------	-----
15. p-Nitrophenol	1-±400	±5.3	------	-----	------	-----
16. 2, 4-Dinitrophenol (1% Na_2CO_3)	1-±200	±2.6	------	-----	------	-----
17. 2, 4, 6-Trinitrophenol (1% Na_2CO_3)	1-±400	±5.3	------	-----	------	-----
18. o-Chlorophenol	1-275	3.7	------	-----	------	-----
19. m-Chlorophenol	1-300	4.0	------	-----	------	-----
20. p-Chlorophenol	1-300	4.0	------	-----	------	-----
21. o-Bromophenol	1-500	6.6	------	-----	------	-----
22. p-Bromophenol	1-500	6.6	------	-----	------	-----
23. o-Iodophenol	1-750	10.0	------	-----	------	-----
24. o-Hydroxy benzoic acid	1-1,000	13.3	1-2,000	18.2	1-2,000	13.3
25. m-Hydroxy benzoic acid	1-±500	±6.6	------	-----	------	-----
26. p-Hydroxy benzoic acid	1-±500	±6.6	------	-----	------	-----
27. Sodium salicylate	1-500	6.6	------	-----	------	-----
28. n-Amyl salicylate (20% alcohol)	1-±4,000	±53.0	------	-----	------	-----
29. Catechol	1-50	0.66	------	-----	------	-----
30. Resorcinol	1-25	0.33	------	-----	------	-----
31. Hydroquinone	1-25	0.33	------	-----	------	-----
32. n-Hexyl resorcinol (30% glycerine)	1-15,000	200.0	1-17,000	154.0	1-20,000	133.0
33. n-Butyl ether of resorcinol	1-1,500	20.0	1-2,000	18.0	1-4,000	26.6
34. n-Amyl ether of resorcinol	1-4,500	60.0	1,7,000	63.6	1-9,000	60.0
35. n-Hexyl ether of resorcinol	1-11,000	146.0	1-17,000	154.0	1-22,000	146.0
36. Phenyl propyl ether of resorcinol	1-7,500	100.0	------	-----	------	-----
37. Chlorothymol	1-8,000	106.6	1-16,000	146.0	1-20,000	133.0
38. "Clymocol"	1-±5,000	±66.0	1-±5,000	±45.0	1-±5,000	±33.0
39. 2-Cl-5-OH-toluene	1-1,000	13.3	1-1,500	13.6	1-2.000	13.3
40. Iodine	1-20,000	266.0	1-20,000	182.0	1-30,000	200.0
41. Mercuric chloride	1-±20,000	266.0	------	-----	------	-----

± "Approximate"; insolubility of the drug made more accurate estimation impossible.

The substitution of halogen atoms in phenols increases their fungicidal ability several times. It is worth noting that both in the case of phenol and thymol, the introduction of a chlorine atom increases the fungicidal power the same amount, namely fourfold. A substituted iodine atom has a greater effect than a bromine atom which in turn produces a higher phenol coefficient than does a chlorine atom. The substitution of halogen atoms also decreases the solubility of the compounds. Again the position on the ring taken by the

substituted halogen atom has no marked effect upon the fungicidal power.

In contrast to the statement that the position occupied by substituted alkyl groups or halogen atoms is unimportant is the fact that a carboxyl group substituted in the ortho position has been shown to exert an effect more than twice as great as when substituted in the meta or para positions. The substitution of the carboxyl group in the ortho position increases the fungicidal power of phenol more than does a halogen atom or short alkyl side chain.

Of the phenol derivatives which have been studied n-hexyl resorcinol and the n-hexyl ether of resorcinol possess the highest fungicidal power followed closely by chlorothymol. All three should prove useful in the treatment of fungous skin infections and all are being subjected to clinical trial. The n-butyl, n-amyl and phenyl propyl ethers of resorcinol as well as 3.5-dibutyl phenol have exhibited sufficient fungicidal ability also to warrant further study. Thymol, carvacrol and salicylic acid are now being used as fungicides and have given fairly satisfactory results in the actual treatment of skin infections.

Fungicidal Power Compared with Bactericidal Power.—A close relationship between fungicidal and bactericidal power has been observed throughout this study of phenol derivatives. The highest dilution of phenol which will kill *M. tropicalis*, (1-75) is very nearly the same as the maximum dilution (1-80) which kills *B. typhosus*. The dihydric phenols, hydroquinone, resorcinol and catechol have coefficients of less than one on both bacteria (*B. typhosus* and *Staph. aureus*)[13] and fungous organisms (*M. tropicalis*). The coefficient (2.5) for p-cresol (*B. typhosus*) reported by Coulthard, Marshall and Pyman[15] compares with the coefficient of 2.5 obtained when using *M. tropicalis*. As the length of the side chain increases, in alkyl substituted phenols, however, the phenol coefficients increase but more rapidly if the tests are made with fungous organisms than when bacteria are used. Thus hexyl resorcinol has a coefficient of 200 with *M. tropicalis*, 154 with Cephalosporium, and 133 with Sporotrichum, all fungous organisms, while the commonly accepted coefficient for the same substance on *B. typhosus* is approximately 46. This difference may be due in part to the use of a different method of calculating the coefficients. An interesting comparison may be made between the results obtained by Klarmann[13] and those obtained in this work with the monoethers of resorcinol.

TABLE II

PHENOL COEFFICIENTS

	B. Typhosus (REPORTED BY KLARMANN)	*Staph. Aureus* (REPORTED BY KLARMANN)	*M. Tropicalis*	*Cephalosporium*	*Sporotrichum*
n-butyl ether of resorcinol	20	18	20	18	26.6
n-amyl ether of resorcinol	38	36	60	64	60
n-hexyl ether of resorcinol	46	125	146	154	146

SUMMARY

1. The fungicidal powers of 37 derivatives of phenol and of two miscellaneous fungicides, iodine and mercuric chloride, have been determined by

using as test organisms *Monilia tropicalis,* Cephalosporium, and Sporotrichum. These organisms were selected after preliminary tests as being typical of a large group of pathogenic yeasts and molds.

2. The position taken by the substituted alkyl side chains and halogen atoms has been found in general to have little effect on the fungicidal power of phenols; on the other hand salicylic acid, a phenol derivative having a carboxyl group in the ortho position, is far superior as a fungicide to the isomeric meta and para hydroxy benzoic acids.

3. It has been shown that there is a definite increase in the fungicidal power of the alkyl phenol derivatives with an increase in the number of carbon atoms of the side chain; that in dihydric phenols the substituted alkyl radical may take the place of a hydrogen atom of the benzene ring or of a hydrogen atom of the hydroxyl group without causing any significant variation in the fungicidal power.

4. The substitution of halogen atoms has been found to increase the fungicidal power from four to ten times; the fungicidal strength increasing from chlorine through bromine to iodine. The introduction of nitro groups into phenols does not increase the fungicidal power.

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LABORATORY METHODS

OBSERVATIONS ON THE TECHNIC OF THE FRIEDMAN TEST FOR PREGNANCY*

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IN A RECENT address Dr. T. B. Magath[1] editor of the *American Journal of Clinical Pathology*, made the following statement: "Although clinicians have scientifically studied the signs of pregnancy for more than 5,000 years, recently devised simple laboratory tests can detect pregnancy earlier and more accurately than any set of clinical observations yet perfected."

The first practical laboratory test for pregnancy was introduced in 1927 by Aschheim and Zondek.[2] It consists of six subcutaneous injections of urine twice daily for three days into five immature mice, followed by inspection of the animals' ovaries one hundred hours after the first injection. The presence of blood points and lutein tissue constitutes a positive reaction. Aschheim and Zondek reported a series of 1,400 tests with 98.5 per cent accuracy. The test has now been performed throughout the world in many thousand cases, and its reliability fully established. The material in the urine responsible for the reaction was called prolan and was considered to consist of two anterior pituitary hormones.

The hypophyseal principle was found by Zondek in the hypophysis, in certain extracts of other organs, in placental tissue, decidual tissue and perhaps most important of all in the urine of pregnant women. A small quantity (about one mouse unit) was found in the urine of nonpregnant females and as much as from 3,000 to 5,000 units in pregnant women. This observation led Aschheim and Zondek[3] to propose the demonstration of a large quantity of prolan in the urine as a test for pregnancy. Although there is some question as to the site of origin and nature of prolan, the evidence seems to favor the view that it is an anterior hypophyseal secretion and that because of the presence of the living placental tissue in the pregnant woman it is formed continuously in very large amounts and excreted in part in the urine.

The pregnancy test was the outcome of animal experiments by Zondek[3] in the study of ovarian function. These studies of Zondek in Germany and those of P. E. Smith[4] in this country in 1926 were the first to reveal that sexual maturity was controlled by the anterior hypophysis and that hormones from this body initiate follicle ripening, ovulation and luteinization.

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In 1929, Friedman[5] recommended the use of a single mature rabbit to replace the five immature mice of the Aschheim-Zondek test. In the rabbit there is no periodic ovulation or luteinization of follicles and these changes normally occur only after copulation, consequently the mature female rabbit isolated from the male for a period of several weeks is an ideal animal for demonstrating the effect of prolan. In addition to the simplification of the pregnancy test by the use of one rabbit, the time required is shortened from one hundred hours to forty-eight hours. The technic suggested by Friedman[8] in 1931 consists in the intravenous injection of 4 c.c. of a voided specimen of urine (three times daily for two days), a total of 24 c.c., into a mature female rabbit previously isolated for at least three weeks. About forty-eight hours after the first injection the ovaries are examined at autopsy. In a positive test the ovaries are enlarged, and show a number of prominent hemorrhagic follicles. In a negative test the ovaries are white, and while ripe follicles 1 to 2 mm. in diameter may be seen these contain clear colorless fluid.

Since the original publication by Friedman[5] a large number of tests for pregnancy, using rabbits as the test animal, have been reported in this country[6, 7] confirming his results.

The results of the 350 tests performed at the Mount Sinai Hospital of Cleveland are shown in Table I.

The earliest positive test in this series was thirty-two days after the last menstrual period.

TABLE I

SUMMARY OF 350 FRIEDMAN TESTS AT MOUNT SINAI HOSPITAL OF CLEVELAND

Total tests	350	
True positives (uterine pregnancy)	92	
True positives		
(intact ectopic pregnancy)	2	
(hydatid mole)	1	
False positives (tumors)	1	+ 2 in males
False positives (tuberculous oophoritis)	1	
True negatives	131	
Not pregnant	69	
Delayed menses	12	
Fibromyoma uterus	10	
Acute pelvic inflammatory disease	1	
Chronic pelvic inflammatory disease	1	
Tuberculous pelvic peritonitis	10	
Carcinoma cervix	7	
Ovarian cyst	4	
Subacute endometritis	3	
Postpartum	2	
Menopause	2	
Ectopic pregnancy (no intact chorionic villi)	2	
Polypoid endometrium	1	
Hydatid mole (postoperative)	1	
Carcinoma breast	1	
Carcinoma ovary	1	
Benign tumor of scrotum	1	
Sarcoma of uterus	1	
Fibromyoma with sarcomatous change	1	
Male urine (200 c.c.)	1	
False negatives	1	
Doubtful (early tests of series)	5	
Experimental	114	

The one false positive and the one false negative test will be discussed later. Tests were done in four cases of tubal pregnancy, two of which were positive and two negative. On microscopic examination of the fallopian tubes intact chorionic villi were present in both cases which were positive while in the negative cases the villi were degenerating or necrotic.

In the case of hydatid mole the preoperative specimen of urine gave a characteristic strongly positive reaction and a negative result was obtained on testing the urine twelve days postoperatively. In the cases of tumors other than hydatid mole three gave positive results and twenty-two gave negative results. Of the three positive tests one was a female with a clinical diagnosis of teratoma of the ovary and the other two tests were in the case of a male with a clinical diagnosis of teratoma of the testis. The urines from the cases of teratoma of the ovary and of the testis were obtained through the courtesy of Dr. D. Seecof of City Hospital, Cleveland. The negative tests included various types of benign and malignant genital tumors as shown in Table I.

Fig. 1.—Gross photograph. Natural size. Rabbit ovaries. Gross appearance of positive test of one, two, three and eight days' duration and of negative test.

OBSERVATIONS RELATING TO THE TECHNIC OF THE FRIEDMAN TEST

Many minor modifications of the technic of the Friedman test have already been reported in the literature.

A. *Selection of Animals.*—Of first importance is the selection of proper animals. Some workers have used animals from three to four months of age. Friedman[8] recommends the use of rabbits after bearing young for biologic assay, as these animals give the most constant response to a given dose of the hormone. For routine use, however, ordinary domestic female rabbits weighing from 2 to 4 kg. give satisfactory results.

As in other biologic tests the rabbits are found to vary considerably in their reaction to a given dose of hormone. In a series of 55 mature rabbits, each of which was injected with 10 c.c. of pooled urine from pregnant women, 49 gave characteristic positive reactions, 4 gave minimal or grossly doubtful reactions, and 2 were negative. One of the two negatively reacting rabbits gave a negative result on repeating the test with a 10 c.c. dose but a positive result on injecting 20 c.c. The other was not retested.

As stated above the rabbits must be isolated from male rabbits or other females for a period of three weeks before injection. Some workers have eliminated this period of isolation by preliminary laparotomy and selection of those animals that have obviously not experienced hypophyseal stimulation for several weeks, with ovaries showing ripe or nearly ripe follicles with clear fluid but no hemorrhagic areas or corpora lutea.

The importance of inspection of the ovaries before selection of the animals was emphasized by Wood[9] who states: "We attach much importance to the preliminary inspection of the ovaries as we feel that this gives us an ideal opportunity for correctly interpreting the actual changes brought about by the anterior pituitary hormone present in the urine. This procedure is in our judgment the best possible control of the test."

We have followed this suggestion in 178 tests and recommend its use as strongly as does Wood.

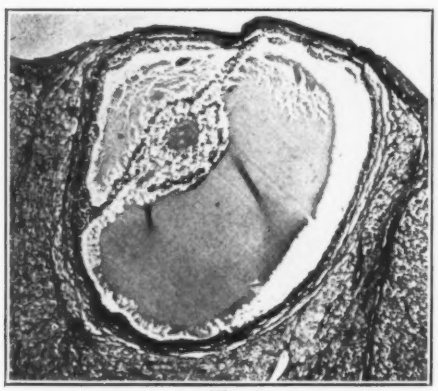

Fig. 2.

Fig. 3.

Fig. 2.—Photomicrograph. Low power. Rabbit ovary. Normal ripe follicle.
Fig. 3.—Photomicrograph. High power. Rabbit ovary. Normal ripe follicle, showing granulosa cells and theca.

Of the 178 rabbits in which preliminary inspection of the ovaries was done, three showed corpora hemorrhagica, seven showed corpora lutea, four showed old areas of hemorrhage, and seven were found to be pregnant. These findings show the necessity of isolating rabbits several weeks or more for the complete involution of corpora lutea before the animal is in a satisfactory condition for the test. In the case of the seven rabbits (recent purchases) which were found to be pregnant on preliminary inspection, the test would have had to be repeated as Friedman has shown that, during the pregnancy, the rabbit requires a larger amount of the hormone to produce corpora hemorrhagica than it does in the non-pregnant state.

Of the 3 rabbits showing corpora hemorrhagica, one had not been used previously and had been isolated for a period of three weeks, the other 2 had been positive about four weeks previously. Of the 7 rabbits showing corpora lutea, 4 were recent purchases, isolated about one week, the other 3 had been

positive from one to two months previously. Of the four rabbits showing old areas of hemorrhage one was recently purchased and isolated only several days, the other three had given positive results, from one to two and one-half months previously.

It will be noted from the above figures that the majority of corpora hemorrhagica and old areas of hemorrhage (the changes which would lead to false positive results) observed from three to ten weeks after hypophyseal stimulation occurred in animals which had been used previously and in which positive results were obtained. In our experience 4 out of 5 rabbits as purchased in the open market were satisfactory upon preliminary inspection for immediate use in the Friedman test. In those as purchased on the market and isolated for the prescribed period of three weeks only one showed a corpus hemorrhagicum and

Fig. 4.

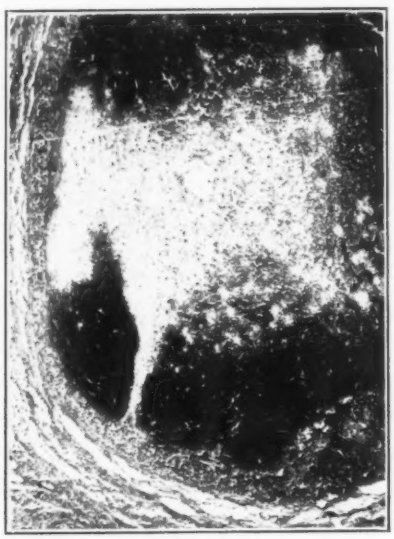

Fig. 5.

Fig. 4.—Photomicrograph. Low power. Rabbit ovary. Positive test of twenty-four hours' duration (corpus hemorrhagicum).

Fig. 5.—Photomicrograph. Low power. Rabbit ovary. Positive test of forty-eight hours' duration (early corpus luteum).

might have given rise to a false positive result. The preliminary operation, therefore, is of particular value in animals previously giving positive results, the period of complete involution in these artificially produced corpora hemorrhagica being variable and in some instances requiring longer than three weeks (see Fig. 10 of subinvoluted ovary).

All of the rabbits were isolated from other rabbits as soon as purchased and were kept in individual pens at all times.

B. *Anesthesia.*—The anesthetic generally employed for laparotomy has been ether, although the use of sodium barbital and avertin[10] and other anesthetics has been reported. In the series of tests reported here morphine sulphate was injected intravenously and found most satisfactory. While the majority of tests were done using a half grain of morphine for rabbits weighing from 2 to 3½ kg.

(the average size of the animals used in the test) doses as high as 2 gr. were employed in the early tests of this series. Within a few minutes after the injection of the morphine, the animal becomes drowsy and relaxed and is ready for operation. Rabbits anesthetized with morphine will show local reflexes with muscular contraction on sharp cutting and quick handling but no pain apparently. These reflexes can be minimized or even abolished if the movements of cutting and handling are deliberate. Rarely the larger animals are insufficiently anesthetized with a half grain of morphine, whereupon an additional quarter grain is given. The animals remain drowsy for several hours and at no time do they show any ill effects. The use of morphine intravenously has the additional advantage of making it possible to perform the laparotomy without an assistant and of being economical.

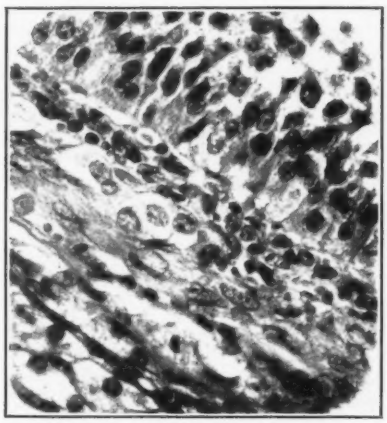

Fig. 6.

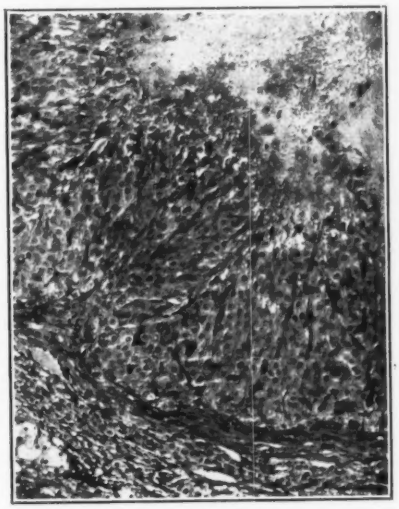

Fig. 7.

Fig. 6.—Photomicrograph. High power magnification of rabbit ovary. Luteinizing follicle showing proliferation of granulosa cells and of theca.

Fig. 7.—Photomicrograph. High power. Rabbit ovary. Corpus luteum of forty-eight hours' duration showing proliferation of capillaries.

C. *Urine for Injection.*—In order to avoid doubtful and false negative results, the urine to be tested must be a satisfactory specimen. The patient is instructed to restrict fluids the evening before the specimen to be tested is collected, to void before retiring and to promptly bring the first voided morning specimen to the laboratory. The urine should be of a specific gravity of 1.015 or more. The amount of urine injected intravenously by different workers has apparently varied more than any other single detail of the test. Friedman[5] first recommended a single dose of 5 c.c. Later[7] he recommended the use of 24 c.c. in six doses of 4 c.c. each. Other workers have used single doses of 10 c.c., 2 doses of 10 c.c. each at twenty-four-hour intervals, and still others have injected concentrates of the hormone obtained by alcohol precipitation. The tests in the first half of our series were done with a single injection of from 10 to 15 c.c. At present we regard 30 c.c. in two doses of 15 c.c. each as more advisable. The

doses are given four hours apart in order to avoid injury to the rabbits by a large amount of urine which is always somewhat toxic.

The use of a large amount of urine does not lead to false positive results, since as much as 100 c.c. of urine from nonpregnant women have been injected in divided doses and the equivalent of 200 c.c. of male urine concentrated by alcohol precipitation have been injected in a single dose without producing false positive results.

In a certain number of cases (3 per cent in this series), the urine on injection proves toxic and results in the death of the animal. The urines in this series which proved toxic were either turbid with bacteria or were obtained from patients who were receiving medication. The urine may be purified before injection into another rabbit by the method recommended by Zondek as follows: The hormone is precipitated by adding five volumes of 95 per cent alcohol to 60

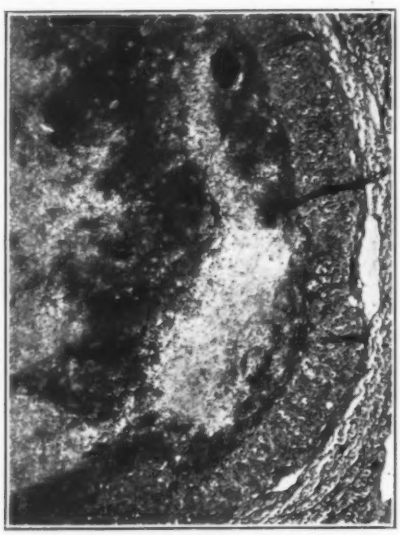

Fig. 8.

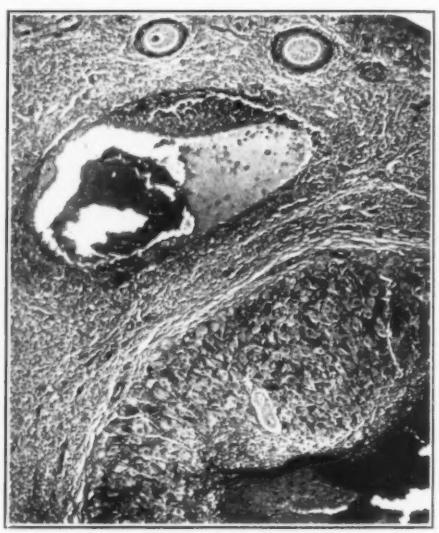

Fig. 9.

Fig. 8.—Photomicrograph. Low power. Rabbit ovary. Positive test of seventy-two hours' duration (corpus luteum).

Fig. 9.—Photomicrograph. Low power. Rabbit ovary. Positive test showing follicles of eight days' and of twenty-four hours' duration. (Corpus luteum, corpus hemorrhagicum.)

c.c. of urine. The precipitate is collected by centrifuging in large tubes, and the supernatant fluid is discarded. The precipitate containing any anterior pituitary hormone present is dissolved in as small an amount of distilled water as possible and extracted by shaking for fifteen minutes with three volumes of ether to remove the toxic substances. Folliculin is also removed by the ether extraction. The ether washings are discarded and the excess ether removed from the solution by aeration. The solution is then made up to about 10 c.c. with distilled water and injected slowly in a single dose. The 60 c.c. volume is chosen to allow for loss of about one-half of the hormone in handling.

D. *Examination of Ovaries.*—While in general one hundred hours is the time interval allowed when using the immature mouse as the test animal, Zondek[11] has reported a method of shortening the time of the mouse test from one hundred

to seventy-two hours. The modification is as follows: A 30 c.c. sample of urine is extracted with three to four volumes of ether. The ether washings are discarded and 0.9 gm. of glucose is added to the urine residue. The usual injections are then given.

In the test using the rabbit, Friedman[5] recommends forty-eight hours, and this is the time interval which has been generally adopted. Normally after copulation, twelve hours are required for the development of corpora hemorrhagica in the rabbit's ovary.

In a negative test the ovaries are white, and while numerous ripe follicles may be seen, there are no areas of hemorrhage present. In a positive test at forty-eight hours the ovaries are large, and show a number of hemorrhagic follicles which are easily recognizable grossly. Microscopically not only is hemorrhage in the follicles striking but there are also a number of apparently recently proliferated capillaries and the presence of one or more layers of maturing lutein cells.

The gross and microscopic examination of the ovaries (see Figs. 1 to 9 incl.) removed at intervals of from one to thirteen days after injection of urine from a

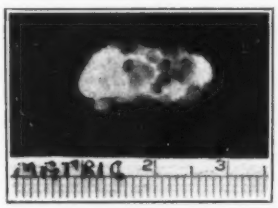

Fig. 10.—Gross photograph. Natural size. Rabbit ovary. Subinvolution of ovary (twenty-eight days after injection of urine of pregnant woman).

pregnant woman show important changes in the development of corpora lutea from the ripe follicles. At the end of twenty-four hours some of the follicles show a small central area of hemorrhage and lutein cells one or more layers in thickness apparently developing from the granulosa cells and from those of the theca interna. There is also visible in some of the follicles evidence of proliferation of new capillaries. It is probable that the hemorrhage in the corpus hemorrhagicum is due not to rupture of the follicle at ovulation but to bleeding from capillaries proliferating to maintain the developing corpus luteum. This view is supported by the fact pointed out by Friedman[5] that corpora hemorrhagica and corpora lutea may be formed whether or not ovulation occurs. In addition to maintaining the corpus luteum newly developed capillaries share in the organization of the follicular hemorrhage. At the end of forty-eight hours some of the follicles show larger areas of hemorrhage and a thickened collar of lutein cells, and quite a number of others show changes characteristic of the twenty-four-hour reaction. At the end of seventy-two hours the follicles show, in addition to more lutein tissue, well-developed evidence of organization of the central hemorrhage. By the fifth day the process is quite distinctive grossly, and at the end of a week the corpora lutea are prominent and unmistakable. Ovaries at five- and

eight-day intervals show follicles which grossly and microscopically can be dated as fully developed one-, two-, three-, five-, and eight-day reactions, indicating that the stimulus of the hormone is exerted continuously for about eight days.

Sections stained with Scharlach R. show a scanty deposit of lipoid globules in the lutein cells as early as twenty-four hours and increasing amounts of lipoid in the older lutein cells. Those of three and five days show abundant globules.

The majority of workers have accepted gross inspection of the ovaries as sufficiently characteristic for a final diagnosis in the test for pregnancy. While in the vast majority of instances we have found it satisfactory, our one false positive reaction would have been avoided by microscopic examination. This animal was injected with urine from a case of extensive tuberculous salpingitis and oophoritis, and forty-eight hours later showed hemorrhagic follicles. The test was repeated in two separate rabbits, both of which showed similar hemorrhagic follicles in the ovary which were grossly indistinguishable from corpora hemorrhagica. Microscopic examination, however, showed that there were degenerative and necrotic changes in the wall with hemorrhage into the follicles but no evidence of lutein tissue formation.

After surgical removal of the affected ovary and fallopian tubes of the patient, the Friedman test was negative on repeated trials.

These findings were further controlled by the injection of urine from five cases of advanced pelvic tuberculosis which we obtained from the Cleveland City Hospital through the courtesy of Dr. S. E. Wolpaw. All of these rabbits gave negative results. In 42 tests subsequent to this error all positive tests were controlled by removing one ovary at laparotomy and examining it microscopically for lutein tissue after frozen section. These operated animals may be used for a second Friedman test in about four weeks.

The one false negative test was obtained in the first half of this series when a single dose of 15 c.c. of urine was used. The specimen of urine had a specific gravity of 1.014. On repeating the test three weeks later, using the same rabbit and 15 c.c. of urine with a specific gravity of 1.028, a positive result was obtained.

Some workers have retested the animals giving negative results in cases for diagnosis with known positive urine. In the light of the variable reaction capacity of the animals this method of control is certainly indicated especially when less than 30 c.c. of urine is injected. In the latter part of our series 29 negative tests were controlled by injection of positive urine and this control of negative tests is now a routine procedure.

Since freshly voided urine from a known case of pregnancy is not always available for injection, the majority of the controls on negative tests in our series were done with concentrated urine. The concentration may be carried out as described above for detoxifying urine. For concentrating large amounts of urine the original procedure has been modified in order to reduce the amount of reagents required. The method is as follows: 500 c.c. of 95 per cent alcohol are added to 1,000 c.c. of pregnancy urine in a shallow pan and the mixture evaporated to dryness by fanning (in a hood). The sediment is taken up in

200 c.c. of 60 per cent alcohol and shaken vigorously in a shaking machine for one-half hour. The material is then filtered, the residue discarded and the hormone is precipitated from the filtrate by the addition of five volumes of 95 per cent alcohol and allowed to stand in the ice box overnight for complete precipitation. The supernatant fluid is discarded and the precipitate is dissolved in as small an amount of distilled water as possible (about 50 c.c.). This solution is extracted with three volumes of ether in order to remove the folliculin. The ether washings are discarded and the solution is aerated to remove the excess ether. An amount of the material equivalent to 40 c.c. of the original specimen is distributed into small tubes, each tube to be used as a positive control for negative tests. This measured concentrate is again precipitated with five volumes of alcohol and stored under alcohol in the ice box ready for use. (The amount equivalent to 40 c.c. of urine was selected in order to allow for some loss of the active principle in handling.) Before a lot of the concentrate is used for controlling negative tests a sample of it is injected into a suitable rabbit to determine its potency. Before the injection into a rabbit the alcohol is poured off and the sediment is taken up in a few cubic centimeters of physiologic saline solution.

RECOMMENDED PROCEDURES FOR THE FRIEDMAN TEST

1. Selection of ordinary mature domestic female rabbits weighing from 2 to 4 kg.

2. Isolation of the animals in individual cages.

3. Use of morphine sulphate intravenously as an anesthetic.

4. Preliminary laparotomy and inspection of ovaries with selection of animals with ripe or nearly ripe follicles but no corpora hemorrhagica or corpora lutea.

5. Injection intravenously of 15 c.c. of clear, filtered, first morning specimen of urine, warmed to body temperature, of a specific gravity of 1.015 or higher. This dose is repeated in about four hours.

6. Forty-eight hours after the first injection the wound is opened and the ovaries are reinspected. If the ovaries grossly appear positive, one ovary is removed and examined microscopically by frozen section. The presence of lutein tissue in the wall of one or more follicles associated with more or less hemorrhage into the follicle constitutes an undoubtedly positive reaction.

7. If the ovaries show no corpora hemorrhagica the wound is closed and the rabbit injected with 15 c.c. of known positive urine or concentrated urine as described above. A tentative report of a negative test is given. Forty-eight hours after the injection of the positive control urine the wound is opened and the ovaries are reinspected. If the ovaries now show corpora hemorrhagica and lutein tissue is observed microscopically, the final report of a negative test with the urine for diagnosis is given.

If facilities are not available for making frozen sections, a reinspection of the ovaries on the fifth or sixth day after injection will show sufficient lutein tissue grossly to make an undoubtedly positive diagnosis possible.

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AN IMPROVED ELECTRODE FOR THE MEASUREMENT OF POTENTIALS ON THE HUMAN BODY*

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IN AN attempt to map the electrical potentials of the various parts of the body in connection with studies of the relationship of such potential differences to metabolism, it was necessary to use a large number of relatively nonpolorizable electrodes. Such an electrode has been described by Alvarez, Freedlander, and Clark[1] and used by Purdy and Sheard[2] in connection with studies of metabolism. Our experience with the Alvarez electrode led us to attempt to eliminate the difficulties which we experienced: clumsiness due to the comparatively large size of the "H" tube; frequent cracking of the cross member of the tube, especially when this electrode is used with noncooperative psychopathic patients; the variation of conductivity due to changes in the amount of moisture in the gauze wicks; and the change of resistance and potential arising from the loosening of the copper wire embedded in stiff copper amalgam.

Our improved electrode, which is drawn to scale in Fig. 1, consists essentially of an outer tube of one inch diameter, which functions in the same manner as the wick-bearing arm of the Alvarez "H" tube, and an inner tube ¼ inch in diameter, which functions essentially in the manner of the second side of the "H" tube. The chemical composition of the electrode is the same as that used by Alvarez and his coworkers, i.e., saturated lead chloride, lead-mercury amalgam (3 per cent to 97 per cent) and copper wire connection. We, however, have omitted the stiff copper amalgam. The small inner tube is bent

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as shown, and a bulb ½ inch in diameter blown in the middle of the shorter arm, in order to give a larger cross-section to the amalgam lead-chloride junction. This design prevents spilling of the mercury in case the electrode is tipped. A graduation, etched on the equator of this bulb, serves to standardize the level of the mercury-lead amalgam.

This inner tube is mounted by thrusting it through one of the holes of a two-hole rubber stopper. A short tube (*B*), placed in the second hole, provides for the filling of the electrode and also serves as an anchor for the copper wire leading off from the electrode (*C*). This wire is run directly into the mercury-lead amalgam, thus eliminating the stiff copper amalgam used by Alvarez and his coworkers. The copper amalgam is inconvenient in assembly, tends to jar loose with use, and is unnecessary to the proper operation of the electrode. A semi-permeable membrane ("fish-skin" condom, washed in alcohol and ether)

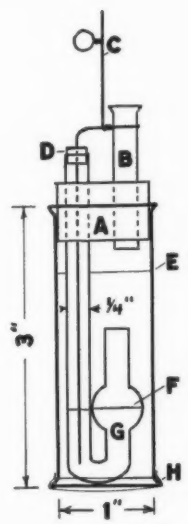

Fig. 1.—Improved electrode. *A*, rubber stopper; *B*, open glass tube; *C*, copper lead-off wire loop for hanging electrode; *D*, sealing wax plug; *E*, level of lead chloride solution; *F*, etched graduation on inner glass tube; *G*, lead mercury amalgam in inner tube; *H*, semi-permeable membrane attached by rubber bands over end of 1-inch tube. The lip of the 1-inch tube allows the electrode to be held loosely in clamp, if desired.

stretched somewhat loosely over the bottom lip of the one-inch tube, furnishes a fairly constant moist conductor, and the slight bulge of the membrane under the weight of the solution in the outer tube gives a means of standardizing the pressure of the electrode on the patient's skin. Our experience indicates that electrode pressure on the skin may affect the potentials which are being measured.

A loop in the lead-off wire (*C*) furnishes a convenient means of hanging a fairly large number of electrodes inside a moist chamber to prevent drying of the membrane and evaporation from the electrode between daily experimental periods.

In actual practice, a single thickness of chamois saturated with lead chloride solution was placed between the electrode and the skin of the patient. It was found convenient to clamp the electrode loosely in a vertical position (with

TABLE I

VARIATION OF POTENTIAL DIFFERENCES DEVELOPED BY THE IMPROVED ELECTRODES

		TIME ELECTRODES IN USE (MIN.)	ELECTRODE NUMBER										AVERAGE MV. VARIATION	AVERAGE VARIATION PER MIN.
			1	2	3	4	5	6	7	8	9	10		
			Potential Variation in Millivolts											
Highest variation	March 6	60	0.60	0.60	0.55	0.50	0.50	0.40	0.30	0.10	0.35	0.15	0.405	0.0068
Freshly prepared electrodes	March 7	50	0.00	0.10	0.00	0.05	0.00	0.05	0.10	0.10	0.05	0.20	0.065	0.0013
Four days later	March 10	38	0.20	0.15	0.20	0.00	0.05	0.10	0.40	0.10	0.20	0.15	0.155	0.0041

patient prone), thus utilizing the weight of electrode rim and of the liquid on the membrane (*H*) as a standard pressure against the skin. The variation of potential differences on three different occasions between each of 10 different electrodes and a single reference electrode is shown in Table I.

The figures in the table represent 3 out of 20 such determinations, which were picked to illustrate the range of potential variation observed. The electrodes were in use on each day for from forty minutes to one hour. The first row of figures shows the variation of potential in millivolts for each of 10 electrodes on a given day and represents the greatest change observed. On this day the electrodes had been in use for a week or more. The second row represents change of potential during a similar period on the next day when the electrodes had been freshly assembled and, therefore, represents the operation of the electrodes under the most favorable conditions. The last row represents the variation of potentials after the same electrodes had been assembled for four days.

The next to the last column at the right of the table gives the average potential variation for the 10 electrodes in each case, and the final column, the average potential variation per minute. Both are in terms of millivolts. It is therefore evident that when the electrodes are freshly assembled, we may expect an average change of approximately 0.001 millivolts per minute, whereas after four or more days' use the electrodes are likely to develop a potential variation of 0.004 millivolts per minute or more. It is therefore recommended that for accurate work with body potentials, the electrodes be freshly assembled every four or five days or whenever a check of potential variation over a period of time shows sufficient change to affect the magnitude of measurements being made.

The figures of Table I were obtained when the electrodes were applied to the human body and under ordinary clinical working conditions, so that temperature of the electrodes undoubtedly varied due to transmission of heat from the body. The variation obtained is therefore not surprisingly large.

SUMMARY

A modification of the Alvarez, Freedlander, and Clark electrodes for use in measuring the potentials of the human body has been developed, as shown in Fig. 1. It possesses advantages over the previous electrode of the same general type: handy size, allowing the use of a large number of electrodes; practically the same nonpolarizing qualities as the Alvarez electrode; uniform pressure on the skin; ease of hanging in solution between periods of use; and comparative ease of assembly, which favors the use of electrodes which are in good condition.

In general we feel that our electrode is much more convenient for clinical use, and especially when used for measurements on psychopathic patients.

For the work of assembling and testing out the electrodes we are indebted to Mr. Joseph Barmack.

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A SIMPLIFIED PSYCHODOMETER*

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THE measurement of reaction time in fractions of a second following drug action in man has offered difficulties to investigators generally. The stop watch is costly and fragile in the hands of students. The split second timer described by Guthrie[1] in 1932, is not readily applicable to visual-mental-muscular reaction time tests. Dr. Metfessel's psychodometer is an excellent reaction timer for such psychophysiologic tests, but it is both complicated and expensive.

To obtain a less expensive, more rugged, and equally satisfactory apparatus for many types of experiments, the accompanying simplified modification of Dr. Metfessel's psychodometer was devised in connection with a study of the effect of caffeine upon the rapidity of mental and physical coordination and reaction in man.

The relatively simple psychodometer is illustrated in Fig. 1. It involves primarily the use of an impulse counter which records time intervals in one-one-hundred-twentieths ($\frac{1}{120}$) of a second. The other parts can be assembled usually from miscellaneous equipment around any physical or physiological laboratory with very little expenditure.

The impulses were furnished by stepping down the 60-cycle alternating current of 110 volts to about 6 volts. Fig. 1 shows the apparatus to be quite readily portable and durable. Both of these factors are highly desirable in laboratory use. In Fig. 2, the schematic diagram of the psychodometer, check lights have been omitted in order to simplify the circuit. It was found that an assistant to the experimenter can easily check the accuracy of the subject's responses in performing the proper act in accordance with the stimulus given. For example, the tapping of the key corresponding to the color of the light stimulus flashed was required in most of the experiments. Auditory or other stimuli could be substituted for light.

The operation of the apparatus is very easy. It requires many more words and much more space to write or tell it than to do it. As the wiring shows in Fig. 2, the closing of a signal switch, for example, "S_2," determines which of the

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possible lights will be flashed when the circuit is closed subsequently by the operator when he throws the double-pole, single-throw switch, "S_4." This latter switch also starts the impulse counter instantaneously with the flash of the light (red in this instance). Upon receiving the visual stimulus, the subject closes the proper key (K_2, in this instance) which stops the impulse counter, but does not put out the light until the operator opens the double-pole switch, "S_4." *Note:* As a matter of fact, in this simplified apparatus without the check light circuit, the tapping of any one of the keys K_1, K_2, or K_3, stops the counter. The subject, however, is not aware of this fact and the operator's

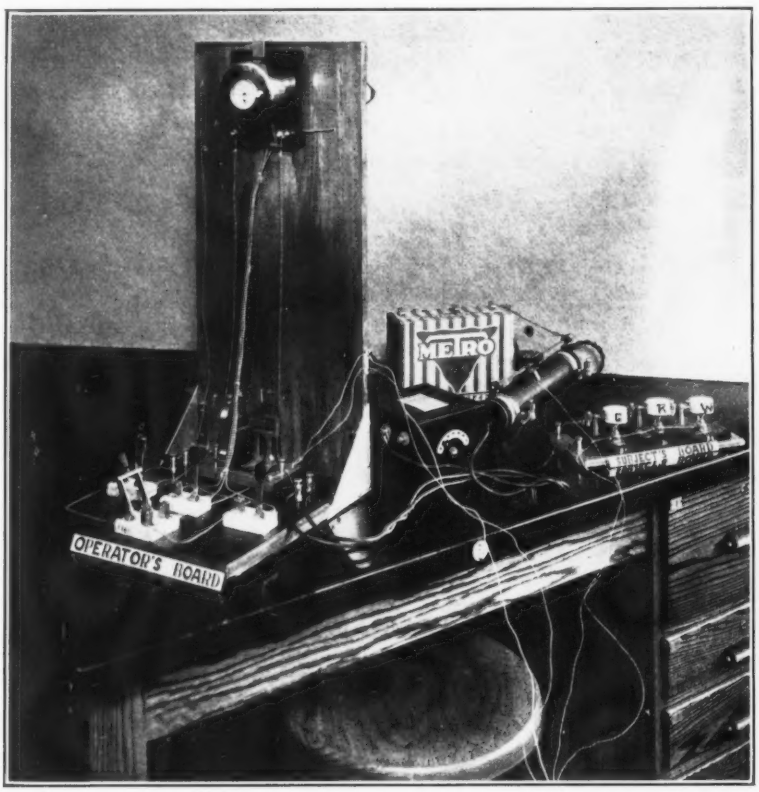

Fig. 1.—Simplified psychodometer for human experimentation.

assistant observes whether or not the subject utilizes the correct key in accordance with the light flashed.

The operator is obscured behind his apparatus board (any large piece of wall-board serves effectively) in order that the subject cannot observe any of the operator's movements. The subject is seated at the opposite end of a 12-foot laboratory table with the subject's tap keys available. Wires of variable length may be used here to allow any desirable distance to intervene between subject and operator. The tap-keys are provided with large wooden discs so that the finger will not readily slip off, as the subject is required to hold the key down temporarily until the operator puts out the light by opening switch, "S_4." The operator's recording assistant is seated at a table near the subject

and in full view of the tap-keys. He notes any errors in the tap-key choice which the subject may make. The subject sits with his right hand on the table in front of the tap-keys and with his left hand resting upon his left knee. In this way all of his movements to the tap-keys are as nearly equidistant as possible.

MANIPULATION

The operator reads the stationary reading of the impulse counter and gives the dial number aloud, which is recorded by his assistant as the original reading for that trial. At variable time intervals after giving an original reading in each case, a light is flashed and the counter starts simultaneously. The subject observes the light flash stimulus, determines its color, and stops the counter by

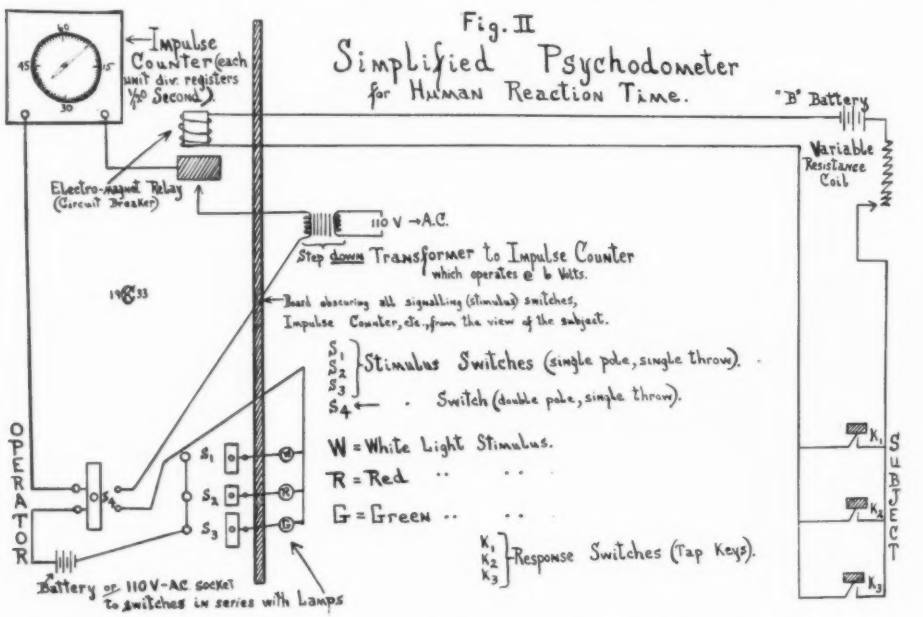
Fig. II
Simplified Psychodometer for Human Reaction Time.
Impulse Counter (each unit div. registers 1/50 Second).
"B" Battery
Variable Resistance Coil
Electro-magnet Relay (Circuit Breaker)
110 V →A.C.
Step down Transformer to Impulse Counter which operates @ 6 Volts.
Board obscuring all signalling (stimulus) switches, Impulse Counter, etc., from the view of the subject.
S1 S2 S3 Stimulus Switches (single pole, single throw).
S4 Switch (double pole, single throw).
W = White Light Stimulus.
R = Red .. ..
G = Green .. ..
K1 K2 K3 Response Switches (Tap Keys).
OPERATOR
SUBJECT
Battery or 110 V-AC socket to switches in series with Lamps

Fig. 2.—Schematic representation of simplified psychodometer for studies in human reaction times.

closing the proper tap-key. The operator then reads the dial number as the final reading for that trial. The difference between the two readings is obviously the total reaction time for this eye-hand mental decision, and muscular response-action phenomenon. Twenty-five trials or more may be averaged for greater accuracy.

The comparisons of reaction time under the influence of various drugs are compared readily, and their effect upon complicated psychophysiologic reaction times recorded quantitatively.

The valuable assistance of Professor Clarence J. Pietenpol of the Physics Department and his assistant, Mr. Ralph L. Hirschel, is gratefully acknowledged.

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300 Pearl Street

A MODIFICATION OF HILL'S RADIOPAQUE MASS FOR THE INJECTION OF LUMINA*

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A RELIABLE technic for the injection of radiopaque materials into the lumina finds many applications in anatomical, pathologic, and surgical studies of lymphatic, vascular, and duct systems. Probably the most satisfactory material for the injection of dead subjects is the bismuth oxychloride mass of Hill[1] when the suspension is properly made. Many workers, however, are unable to obtain consistently good results using the Hill preparation be-

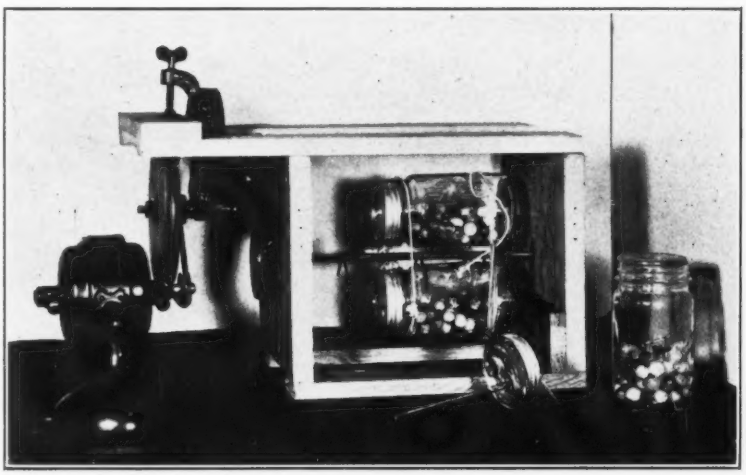

Fig. 1.—An improvised jar or ball mill showing two fruit jars mounted in place and containing marbles. Speed, 10 r.p.m. A jar with a top adapted for injection is shown.

cause they cannot or will not follow the rather troublesome method of preparing the mass. A simple, easily duplicated method for the preparation of a uniform product has been evolved and will be described.

PREPARATION OF THE SUSPENSION

The proportion of acacia to bismuth oxychloride taken is as one is to two. The quantity of water varies with the density of suspension desired. The unmixed ingredients are placed in a glass fruit jar or ball mill crock one-third filled with ordinary glass marbles. This is placed on a ball mill (Fig. 1), such as is commonly used by chemists for grinding materials extremely fine, and is allowed to grind for two to three days. These suspensions are still unstable and will settle out appreciably after standing several days, and

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so it is advisable to regrind for an hour or two before making injections. Table I illustrates certain suspensions and designates their use.

TABLE I

ACACIA GM.	BiOCl GM.	WATER C.C.	TOTAL VOL. IN C.C.	PERCENTAGE BiOCl	GM. BiOCl PER C.C.	SUITABLE FOR INJECTION OF:
50	100	450	500	16.5	0.2	Lymphatics
110	220	500	600	26.5	0.37	Arteries and capillaries
165	330	500	650	33.2	0.51	Arteries

INJECTION TECHNIC

a. *Lymphatics.*—Lymphatic injections are usually made with a Luer syringe fitted with a 27 gauge needle.

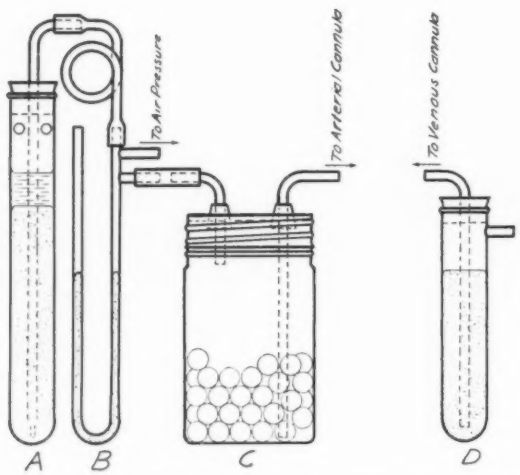

Fig. 2.—Injection system. *A*, Pressure regulator containing mercury topped with water to eliminate mercury spray. The inner tube can be raised or lowered so as to give the desired injection pressure. *B*, Mercury manometer. *C*, Fruit jar used in the grinding on ball mill shown with a top adapted for injection. *D*, Pressure regulating overflow trap containing mercury. The inner tube is adjustable to give the desired pressure in the venous tree. To be used only when visualization of the venous bed is required.

b. *Arteries.*—Arterial injections are best made by cannulating the artery and injecting under constant pressure using the system illustrated in Fig. 2, *A*, *B*, and *C*. The injection pressure is important and will vary with the density of the suspension, the end-result desired, and the species of animal injected. The small arterioles and capillaries are easily ruptured when a low density mass is injected under high pressure. The proper pressure for injection is readily determined and is best done by beginning the injection at a low pressure and then increasing the pressure in steps of 10 mm. of mercury, taking roentgenograms at each level of pressure. The injection is usually complete within five minutes. The interval of pressure throughout which satisfactory pictures are obtainable is sufficient that once it is determined for a species, it will hold for all individuals of this species (Figs. 3 and 4). Likewise, this is a good procedure to follow when injecting a valuable specimen.

c. *Veins.*—Simultaneous injection of arteries and veins produce confusing results because of the inability to differentiate between the arterial and venous systems. However, if the initial injection is made with a low density mass at a low pressure, a satisfactory outline of the arteries is obtained. Then, by increasing the pressure of injection and retarding the escape of the excess mass from the venous side by causing it to flow through a pressure regulating trap, Fig. 2, *D*, a roentgenogram of both arterial and venous beds may be

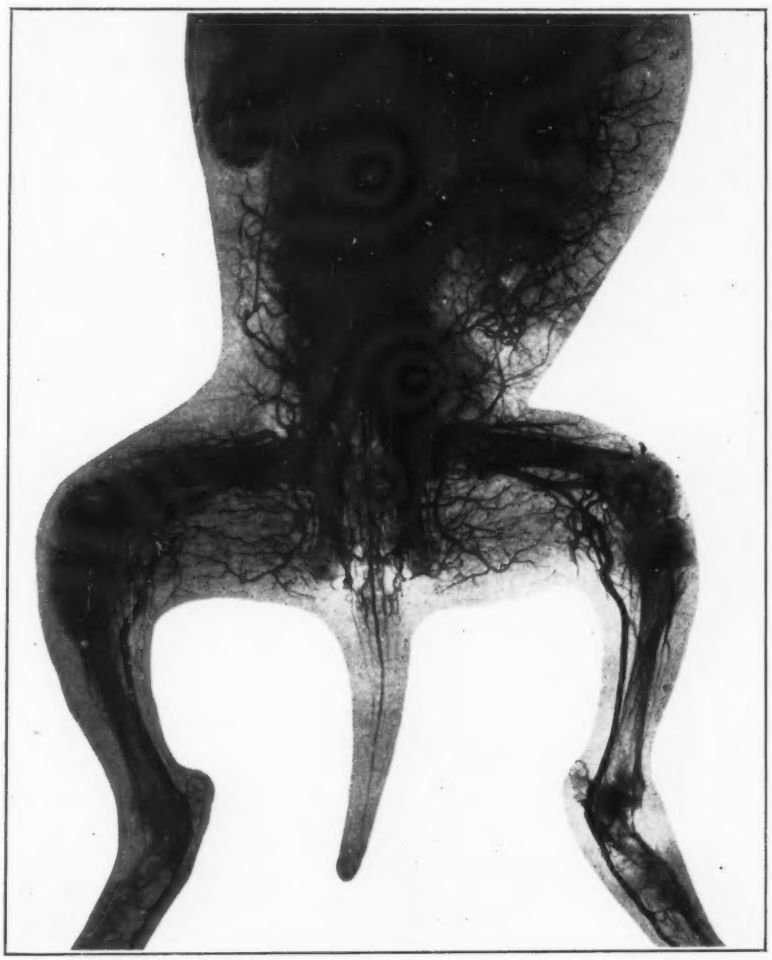

Fig. 3.—A photographic reproduction of a blocked-out roentgenogram of a rabbit injected at 120 mm. Hg pressure with a mass containing 0.5 gm. of BiOCl per c.c. No retouching has been done. Same animal as is shown in Fig. 4. The smaller vessels are lost in the reduction.

secured. Finally, a suspension of starch in acacia solution is substituted for the bismuth oxychloride mass, and the opaque material in the arterial tree is displaced. Since the starch suspension will not pass through the capillaries, a high pressure, 150 mm. Hg, is used in this injection. This will force the opaque mass out of the arteries and permit visualization of the venous bed without the interference of arterial shadows. All roentgenograms should be made while the injection pressure is maintained.

The best results are obtained when the injections are made immediately after death and before the blood has coagulated. If the blood has coagulated, the injection can be done after the clot has retracted and the serum separated.

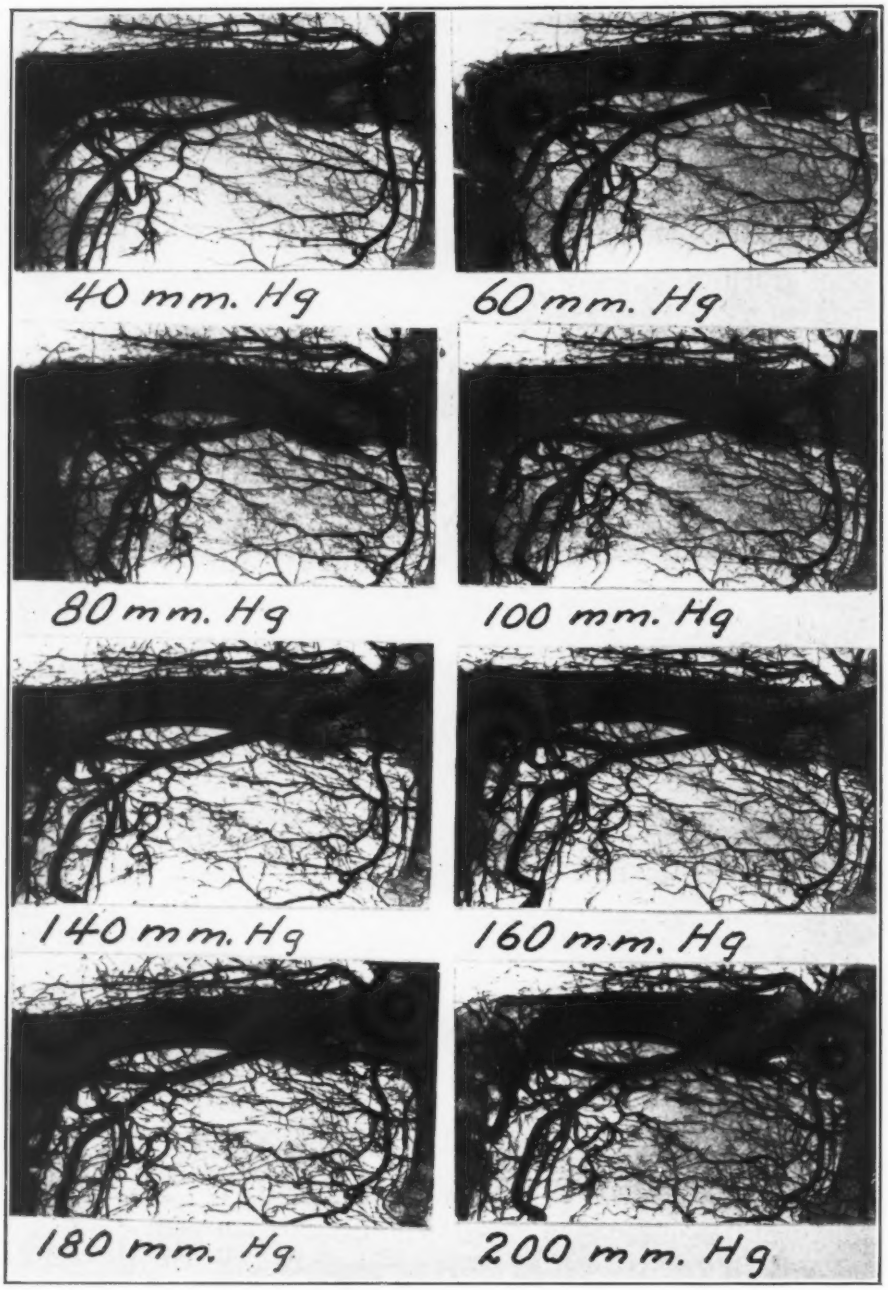

Fig. 4.—Right thigh of animal shown in Fig. 3. Injection pressure as indicated under each successive cut. These are contact prints from roentgenograms which have not been retouched. No vessels show rupture until a pressure of 200 mm. Hg is used; BiOCl extravasated into femur. Stereoscopic roentgenograms clearly show that the nutrient vessels of bones are visualized when injected at a pressure equal to the normal blood pressure of the animal.

With a little experience the proper density of suspension and the correct pressure for injection are readily selected for the problem at hand.

DISCUSSION

Bismuth oxychloride makes a very satisfactory substance for injection, not only because of the high atomic number of bismuth and its consequent opacity to x-radiation, but, also, because of the physical properties of the compound. The bismuth oxychloride* used in this work is extremely finely ground and will assume very definite physical properties when suspended and ground in acacia solutions. After grinding such a suspension for some hours in a jar mill the particles arrange themselves in chains less than ½ micron thick and from 3 to 4 microns long. Upon standing for fifteen minutes the chains arrange themselves into spherical rosettes about eight microns in diameter. No larger particles are formed regardless of the density of the suspension, except on long standing.

The greatest density of any mass suggested by Hill contains 0.4 gm. of BiOCl per c.c. and, as he states, this is a paste. By the method of preparation presented here, the most satisfactory mass for arterial injection contains 0.5 gm. per c.c. and has the consistency of a thin cream. Injection of this material at a pressure equal to the normal blood pressure of the animal usually gives satisfactory visualization of the arterial tree extending out to minute arterioles without the fogging attendant upon capillary shadows.

With a minimum expenditure of effort, exacting no individual skill, the mass described can be prepared, and it will have uniform and reproducible composition and physical properties.

SUMMARY

1. A simple, easily duplicated method for the preparation of a uniform, radiopaque mass, which insures complete injection of lumina, is presented.

2. An injection technic is described.

3. Methods for obtaining visualization of both arterial and venous beds are outlined.

REFERENCE

1. Hill, E. C.: A Radiopaque Bismuth Suspension for Anatomical, Histological and Pathological Research, Johns Hopkins Hosp. Bull. **44**: 248, 1929.

*Merck's C. P. Bismuth Oxychloride, which conforms to "Standards of Murray." See *Standards and Tests for Reagents and C. P. Chemicals*, New York, 1927, D. Van Nostrand Co.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

THYMIC HYPERPLASIA: Follow-Up Study of 30 Cases, Wadlbott, G. L., and Anthony G. E. Am. J. Dis. Child. **47**: 34, 1934.

Of a group of 102 cases in which the diagnosis of status thymicolymphaticus was made by various pathologists, 34 were selected in which no adequate cause of death had been determined. Sixty-eight cases were excluded since other conditions, such as hyperthyroidism, birth trauma, gastroenteritis, and respiratory infections, were associated with lymphoid hyperplasia, and in themselves were sufficient to cause death.

In 11 of the 34 cases death occurred without previous illness, and there were no pathologic lesions to indicate the presence of a previous illness. In 16, minor, usually nonfatal, incidents preceded death. In 7, a syndrome developed manifesting dyspnea, stridor, fever, and shock.

The lungs of all these patients presented uniform changes, characterized by capillary congestion, extravasation of blood cells, and edematous fluid. These lesions alternated with areas of emphysema and atelectasis. In 17 cases petechial hemorrhages were present, involving the heart, pleura, lymph glands, and various other viscera. In some, there were dilatation of the right side of the heart, degenerative changes in the liver, and edema and capillary congestion in other organs than the lungs. Hypoplasia of the suprarenal glands and hyperplasia of the lymphoid organs, as noted by other authors, were present.

Comparison of this pathologic process with that reported in anaphylactic shock in man reveals a close resemblance, if not a complete identity, of the two conditions. Eosinophilia of the tissues and an allergic family or personal history point further to this conception.

On the basis of these findings, it is believed that death may be the result of a primary anaphylactic edema of the lungs and ensuing asphyxiation. Such a theory can be upheld only if one assumes: (1) that anaphylactic shock may occur from incorporation into the body of nonprotein substances; and (2) that absorption of shock-producing antigen may take place by ways other than by injections. Evidence is presented, both from personal experience and that of others, which tends to confirm these facts.

BLOOD LIPIDS, in Children With Scarlet Fever and Rheumatic Disease, Kaiser, A. D., and Gray, M. S. Am. J. Dis. Child. **47**: 9, 1934.

Values for blood lipids in 29 normal children between the ages of five and sixteen were fairly constant, but were considerably below the accepted averages for normal adults.

Values for blood lipids in 43 children convalescing from scarlet fever were practically the same as in normal children. Deviations from the mean were considerably greater, especially for cholesterol.

Similar values for lipids were found in 22 children with acute rheumatism and in 20 with chronic rheumatism. The standard deviations were much greater for the children with rheumatism than for the normal children.

Values for blood lipids deviated considerably from the normal in nephrosis and in nephritis.

Values for blood lipids were not influenced by ordinary variations in diet. The deviations from the mean noted in normal and in sick children seemed to bear no relationship to an increased or diminished intake of fat and of carbohydrates.

No appreciable difference was noted between determinations made on boys and those made on girls. Similar deviations were found in both sexes.

Determinations made in the summer months were uniformly lower than determinations in the winter months.

Studies on the blood lipids of children with rheumatic disease and with scarlet fever revealed values similar to those found in normal children. The standard deviation from the mean was considerably greater in the children with these infections than in the normal children. It is quite likely that the infection, rather than the varied amount of fat ingested, was responsible for the increased deviations.

SEMEN: A Differential Stain that Advances the Study of Cell Morphology, Cary, W. H., and Hotchkiss, R. S. J. A. M. A. **102:** 587, 1934.

The gross examination includes a notation of the amount, which, when complete, and from men under forty years of age, should exceed 3.5 c.c. Lesser amounts should arouse suspicion of deficiency and premature weakening by vaginal acidity, otherwise negligible. Extended periods of sexual abstinence may impart a slightly yellow tinge to the normal grayish opaqueness. Normal specimens commonly show a slight degree of viscosity, which is easily demonstrated by slowly expelling a drop of semen from a pipet. Absence of viscosity with a lessening of opaqueness points to reduced cell content, although the reverse deduction does not hold.

An alkalinity giving a pH of from 8.1 to 8.4 has been so constant that it seems an unnecessary refinement except in research study.

Two specimens, one thick and one thin, are promptly prepared for microscopic examination. This is done by gently protecting a full drop and a fractional drop with cover glasses, the former permitting a gross impression of the density and motility of the cellular content, the latter allowing a preliminary study of individual cell morphology. In the thicker preparation, semen of relatively high fertility presents a field teeming with spermatozoa, many of which are rapidly propelled by fast-whipping tails. While universal cell activity would constitute the ideal, one usually notes in comparatively vigorous specimens that these fast travelers are colliding with a certain number of inactive (25 per cent average) and a variable number of sluggish and impotent cells, which gradually sink toward the lower strata of the field. Familiarity with this picture under standard, dry, high power magnification constitutes the measure by which motility is judged. It is a practical observation that a motile field, though definitely subnormal, is apt to convey a favorable impression on the inexperienced or occasional examiner, but reverse errors in interpretation are rarely made. In the author's experience the practical aspects of motility are that a large number of highly motile cells are essential to fertility; and when a specimen of normal quantity and rich cellular content shows 25 per cent or more of these dynamic cells, a rating of relative fertility must be assumed unless strongly disproved by other indexes to be described. Such specimens usually meet other major requirements of fecundity.

The following technic permits illustration of the head, midsection, and tail of the sperm.

A. Prepare thin cover slip smears as used in the preparation of blood for staining.

B. Fixation in Schaudinn's solution:

1. Immerse for one minute in 7 per cent solution of corrosive mercuric chloride, 2 parts, and absolute alcohol, 1 part.

2. Immerse for one-half minute in 50 per cent alcohol.

3. Immerse for one-half minute in distilled water, 3 ounces, and tincture of iodine, 2 drops.

4. Wash in tap water.

C. Staining Process:

1. Immerse for one-half minute in aqueous solution of eosin, 5 per cent.

2. Immerse for one minute in 50 per cent alcohol, 3 ounces, and concentrated hydrochloric acid, 2 drops.

3. Wash in distilled water.
4. Immerse for two and one-half minutes in hematoxylin.
5. Immerse for one minute in distilled water, 3 ounces, and glacial acetic acid, 2 drops.
6. Wash in distilled water.

Dry and mount.

In appraising seminal specimens, deduct the percentage of abnormal forms found from the total cell count. The resultant figure, when correlated with other data, is of great importance in the more accurate evaluation of the semen. For instance, 40 per cent of inactive cells with 25 per cent abnormal forms would indicate more serious impairment and need for greater improvement in a specimen with a count of 60,000,000 or less than would obtain with a cell count of 100,000,000.

ENTERITIS: Acute, in Infants and Young Children: Bacteriologic Studies, Cooper, M. L., Keller, H. M., and Johnson, B. Am. J. Dis. Child. **47:** 388, 1934.

The bacteriologic methods presented were used in the isolation of streptococci from 65 per cent of 46 infants and children with acute enteritis.

Morphologic and cultural characteristics of these streptococci alone were found to be insufficient for identification.

Marked similarities in the fermentations of carbohydrate were noted in the streptococci isolated from the involved intestinal tracts of patients at necropsy, from the stools of patients, from the throats of patients, and from the intestinal tracts of animals which had received injections.

All the cultures were insoluble in bile with the exception of two, which were questionably so.

For the reasons stated it is suggested that the term *Streptococcus micro-apoikia* (small colony) be employed to designate this group of microorganisms, and that the term *Streptococcus micro-apoikia enteritidis* be employed to designate the strain responsible for the production of enteritis in the patients who were the subjects of this study.

BLOOD, Studies of in Normal Pregnancy, Dieckmann, W. J., and Wegner, C. R. Arch. Int. Med. **53:** 188, 1934.

Previously reported hemoglobin, hematocrit, and erythrocyte values in pregnancy are, in many instances, at variance with each other, and the results are inconclusive because, with but few exceptions, the determinations were made with methods and apparatus which were not standardized, and they were not made on the same women throughout pregnancy. They are of value only in that they indicate the direction of the change.

Determinations of these constituents and of the blood volume were made on various groups of women for the different periods of pregnancy. While the means show a decrease in each substance, reaching a minimum in the last ten weeks, calculation of the difference between the means demonstrates that the change is of no significance.

Similar studies in which the same women were followed throughout pregnancy and the puerperium indicate that the following changes occur in the blood:

There is a definite decrease in the hemoglobin per hundred cubic centimeters of blood. The maximum decrease, amounting to 15 per cent, is from the twenty-sixth to the thirty-fifth week. At two weeks postpartum the hemoglobin is 17 per cent, and at eight weeks it is 14 per cent, below normal.

There is a slight but definite decrease in the hemoglobin per kilogram during pregnancy, and a more marked one after delivery. Apparently there is an attempt during pregnancy to compensate for the increase in tissue by increasing the hemoglobin, but postpartum the stimulus is gone, and there is a lag in the production of hemoglobin.

The changes in the hematocrit value are similar to those in the hemoglobin content. The maximum decrease during pregnancy is 14 per cent. The recovery after delivery is more rapid, although at eight weeks postpartum the hematocrit value is still below normal.

The changes in the erythrocytes are also similar, but the count is normal at three weeks postpartum, thus demonstrating the ability of the hematopoietic tissue to produce red cells but its inability to stock them with the normal amount of hemoglobin.

The total amount of hemoglobin shows an average increase of 13 per cent, but individual cases show a marked gain during pregnancy and a retention of much of the gain postpartum.

The total cell volume shows an average increase of 20 per cent, which is even greater in many individual cases, and a retention of many of the cells postpartum.

The increase in plasma volume is definitely greater than the increases in cell volume and hemoglobin. Therefore, these constituents show a relative decrease and an absolute increase.

Since the erythrocyte is only a vehicle for transporting hemoglobin and since cell counts in pregnancy are often misleading, more time and care should be devoted to hemoglobin determinations.

In pregnancy a hemoglobin content under 10 gm. per hundred cubic centimeters of blood should be diagnostic of anemia.

The changes of blood volume, cell volume, and hemoglobin are a part of the mechanism by which the body is able to take care of its own increase in tissue and the metabolism of the fetus, with the expenditure of the least amount of work.

ANEMIA, Etiology and Treatment of in Pregnancy, Strauss, M. B. J. A. M. A. **102**: 281, 1934.

The "physiologic anemia" of pregnancy is only the effect of hydremia.

The hypochromic anemia of pregnancy is due either to a direct dietary deficiency or to a deficiency conditioned by gastric anacidity, hypoacidity or associated defects in the presence of the fetal demand for blood-building materials. It may be completely relieved, either during or after pregnancy, by the administration of iron in suitable (usually large) doses.

The macrocytic anemia of pregnancy is generally due to a temporary lack in the gastric juice of a specific intrinsic factor, which has been shown to be absent from the gastric juice of patients with Addisonian pernicious anemia in relapse. The ultimate complete return of this factor after delivery is hypothecated. In other cases the lack of the extrinsic factor (associated with vitamin B) from the diet may produce similar effects. The macrocytic anemia of pregnancy ordinarily may be completely relieved with liver extract although iron is frequently required in addition.

There is some similarity of the etiologic mechanism involved in the hypochromic anemia of pregnancy and idiopathic hypochromic anemia in the absence of pregnancy, and in the macrocytic anemia of pregnancy and Addisonian pernicious anemia.

The development of anemia in pregnancy may best be prevented by supplying the pregnant woman with an adequate intake of blood-building materials.

SYPHILIS, Transference of Inguinal Glands in Human, Lunsford, C. J. and Day, P. W. J. A. M. A. **102**: 448, 1934.

The results obtained from the intratesticular inoculation of rabbits with human material from 100 cases of syphilis with a 4-plus Wassermann reaction were as follows:

In 8 cases of primary syphilis 100 per cent of the gland transplants caused positive results in live rabbits.

The glands of 24 patients with chronic syphilis who were under treatment caused negative results in rabbits.

In 38 per cent of 29 cases of untreated latent syphilis, the results in rabbits were positive.

For 6 patients with untreated dementia paralytica inoculations into rabbits gave negative results.

The glands of 12 of 38 patients previously treated for chronic syphilis gave positive results. Six of the 12 patients had received intensive treatment, averaging fifty-two injections of neoarsphenamine and forty-six of mercury.

Three patients with acute syphilis who were adequately treated according to present standards showed live *Spirocheta pallida* in their inguinal glands after a lapse from treatment extending from three to five years. The conditions were diagnosed early; the patients were treated intensively and later were pronounced cured on the evidence of completely negative clinical and serologic findings.

It is concluded that:

1. The diagnosis of primary syphilis by means of gland puncture can be made in 100 per cent of cases.

2. It is probable that patients with late syphilis are not a menace to others while undergoing active treatment.

3. A comparison of the 31.5 per cent positive results obtained in previously treated patients with syphilis with the 38 per cent positive results obtained in untreated patients indicates that, from the standpoint of the biologic cure of the patient, inadequate treatment is very little better than none at all.

4. According to our findings, *Spirocheta pallida* is not present in the cerebrospinal fluid.

5. There is a necessity for frequent reexamination of patients with syphilis, even though it appears that they have been adequately treated according to modern standards.

6. The clinical and serologic tests used as criteria of cure in syphilis are inaccurate and must be revised with increasing knowledge of the disease.

TISSUE, Traumatic Epithelial Cysts of the Skin, Wien, M. S., and Caro, M. R. J. A. M. A. **102**: 197, 1934.

Traumatic epithelial cysts develop as a result of injury to the skin, usually produced by a blunt or tearing instrument. Such cysts occur most often on exposed sites, such as the fingers and palms, and are especially prevalent in those occupations which predispose to injury.

The origin of the cyst is most probably from a bit of epidermis which has been torn from the surface and implanted in the corium. It may also originate from deeper epithelial structures in the absence of surface injury. Here the cyst may form about a foreign body by proliferation of epithelium from the hair follicles or the glandular structures of the skin. Occasionally a foreign body granuloma with cyst formation may simulate an epithelial cyst of traumatic origin.

LEUKEMIA, Monocytic, Leukemic Reticulo-endotheliosis, Foord, A. G., Parsons, L., and Butt, E. M. J. A. M. A. **101**: 1859, 1934.

Leucemic reticuloendotheliosis (monocytic leucemia) is a distinct condition characterized by the presence of a clinical picture similar to that in other acute leucemic states and the presence of an extraordinary number of monocytes in the blood, usually immature forms. Pathologically, a diffuse enlargement of the lymph nodes, spleen, and liver is found, and in the microscopic examination of the tissues, widespread proliferation of reticuloendothelial cells is found, to a degree as great as or greater than is usually found in the so-called infiltrates of other leucemias.

A report of four cases conforming to the foregoing feature, with autopsies in two and biopsy in one, is given.

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EDITORIAL

Have Hospitals Attained a Standard of Mediocrity?

NOT so very many years ago there were numerous hospitals, many of them relatively large institutions, in which the standards of medical practice and the type of care given to patients were notoriously far below what should be and ought to be expected and demanded.

Realizing this situation—and, occasionally, it was very bad, indeed—the American Medical Association and the American College of Surgeons inaugurated a program of hospital standardization whereby a minimum standard of efficiency was formulated to which all hospitals must conform to be "approved." The teeth in this edict lay in the fact that no unapproved hospital was deemed suitable for the training of resident physicians without whom few hospitals can function.

As a result, there are today very few hospitals which have not made such changes as were necessary to secure approval, which does not, however,

signify that all "approved" hospitals have now reached and maintain a high standard of efficiency.

If there be, as probably there are, those who doubt this statement, their attention may well be directed to the remarks of Dr. H. A. Christian, Professor of Therapy and Practice of Medicine in Harvard University Medical School.

In the course of an address delivered before the Congress of Medical Education, Medical Licensure, and Hospitals in 1931[1] he said:

"Present efforts at hospital standardization have attained a fairly satisfactory level of mediocrity for hospitals. A few, often perfunctory, changes in past practices of a hospital now will secure for it a Class A rating. The very poor hospital has been forced to improve, and this is the only real good that has resulted from all the work of those organizations which have been concerned in the classification and rating of hospitals.

"Many institutions, instead of receiving vigorous stimulation for betterment, are basking in the calm complacency of Class-A-dom. Regulations of the classificationists are cheerfully omitted in the case of the best hospitals in the realization, perhaps the subconscious realization, of the mediocrity of their standards and the probable deterioration that would result in these hospitals from their uniform enforcement.

"If there is to be any official standardization of hospitals new standards should be set. At present far too many hospitals are rated as satisfactory, that is, are Class A hospitals, for such rating to have much value or to be any real stimulus to improvement. We are too much in the condition of a heaven peopled by perfect beings, the angels. Fewer hospitals should be Class A than at present is the case; in other words, there should be fewer angels."

That these comments are not without some basis the impartial observer will not dispute.

It may be true that monthly staff meetings are held, as required, but how often are they inspiring and how often do the reports of services lead to critical debate or constructive criticism? How often, on the contrary, do they degenerate into mutual admiration societies?

Constructive criticism, debate which leads to study or to fruitful thought should never be painful nor reluctantly received for, as Herbert Spencer wrote:

"Mankind in its progress passes through three stages: the unanimity of the ignorant; the disagreement of the inquiring; and the unanimity of the wise."

Hospital records alone, now proudly displayed by all "approved" hospitals, furnish many startling and at times amusing illustrations of perfunctory emphasis upon ridiculous detail and equally perfunctory compliance with the requirements of hospital standardization.

The purpose of the hospital record is obvious and elementary: to supply for future contingencies a clear, succinct recital of events from which a clear reconstruction of the situation can be formed in the mind of the reader. A story so plain, so complete, yet without verbosity, that any one can readily understand why such a diagnosis was formulated, and just why a particular

method of treatment was followed, or a specific operation was necessary. The record need not be voluminous but it should be thorough, clearly phrased, and complete in its data of a particular case.

Let those who have attempted to utilize hospital records in the compilation of statistical data or the preparation of papers testify if the majority of records in the majority of hospitals conform to the requirements listed.

The following editorial from an official bulletin of the Philadelphia County Medical Society offers these comments:

"Hospital records are voluminous and cover everything that can be discovered concerning the patient, but in many instances are shy such minor details as to which arm or leg or eye was operated upon, why the operation was done, how the operation was done, and an exact statement of the degree of restoration to normal on discharge. These details, when omitted, are filled in from the fertile imagination of the medical attendant at large. It is seldom, indeed, that any hospital record cites the indication for a first operation and much rarer that it mentions the indication for a second operation. On the other hand, the emotional variations of the midnight hours are engrossed for posterity with infinite care by the night nurse in charge, and the marital affairs of the blood cells and bacteria set down with mathematical accuracy bearing unanswerable testimony to the fact that the technician, reached at least the eighth grade."

That examples may be found, and readily, to justify these strictures will require no extensive investigation. Few, indeed, are the "histories" which could be used verbatim in a scientific report.

It may well be suggested, with all due respect to the "approving" organizations, that in the inspection of records, it might be advantageous, not merely to tick off the fact that records are made and filed but to ascertain exactly *what kind* of records.

It is quite true that standardized records are practically a sine qua non in standardized hospitals, that they are inevitably or generally, therefore, good records is, to speak mildly, open to grave doubt.

Record inspection will invariably present an orderly array of neatly bound sheets which, through their number, their variety, and the stereotyped marginal printed guides, tacitly make claim to the desirable and admirable quality of thoroughness.

And yet one of those responsible for their existence in later years has this to say of them:[2]

"In a medical ward of a class A teaching hospital I recently saw a Jewess, aged forty-five years. Five minutes' conversation brought out the facts that she had always been in reasonably good health until after the death of her husband a year ago; that she then looked hopefully for support from her eldest son; but that about three months ago she gradually experienced the final and crushing conviction that his talents were limited to the selling of newspapers, which yielded a profit of less than a dollar a day. She therefore, in addition to caring for her home and the younger children, took employment in a restaurant, standing eight hours a day washing dishes. Then came backache, sleepless nights of worry, anorexia, loss of 20 pounds, nervousness, utter

exhaustion, and hospitalization. Cursory examination revealed only the ordinary effects of such a life, including possibly some thyroid disturbance.

"Now, I ask of you sticklers for form and order, what do you suppose that woman's folder contained? Five and one-half closely written pages of matter comprised under twenty-eight captions, all neatly underlined with red ink and ruler! Figure out the time that probably took, and then ask yourselves how much time and energy remained to devote to the clinical problem of that woman. We toil through those five and one-half pages in search of useful bits of information. Here and there we find a few, fragmentary and uncorrelated. In the place for 'social condition' is stated that she is a widow; under 'occupation' it is stated that she is a 'housewife'; under 'marital history' that she has four children, but not a word about that fiasco of the eldest son. The paragraph on 'habits' speaks of loss of weight but gives no hint of the possible cause. Breathlessly we work down to the captions 'complaint,' 'onset of present illness,' and 'course of present illness' and find only some sketchy references to pains in the back, palpitation, breathlessness on effort, gas in the stomach, and so on, but never a word of the restaurant or the thoughts in the poor woman's head. Then comes the sacred array of paragraphs on the various systems, with reiteration of shortness of breath under 'cardiorespiratory system,' of stomach gas under 'gastrointestinal,' etc., etc.

"The writer of this history was evidently painstaking and industrious, and yet what a mess he made of it! There is not the slightest doubt that if, before he ever set foot in the medical school, he had been confronted with this patient and had been asked to write down what he could find out about her condition, he would have done incomparably better. And as a commentary on the teaching of clinical history taking is not that the height of irony? The reason for this enormity is obvious. The writer of the history has been so occupied in constructing and polishing the frame in order to meet the standard specifications that he has been unable to paint the picture; indeed, he has scarcely seen the patient and her experiences at all.

"This case, to be sure, is worse than many of our hospital clinical histories, but it is none the less a good illustration of a valid general criticism of unrestrained standardization; namely, stereotypism, perfunctoriness, mediocrity. And, parenthetically, the criticism is applicable beyond the subject of clinical records. I have heard members of a hospital staff oppose betterments in organization for the reason that the hospital was already accredited by the American College of Surgeons and the college did not require the change."

Criticism is most valuable when constructive and the suggestions of the same author are therefore worthy of interpolation:

"1. That we abandon the effort to amplify records for the purpose of future statistical researches except for specific objectives in institutions where there is a reasonable prospect of continuity and reliability of data to justify the effort.

"2. That teachers and those who presume to examine hospitals for accrediting purposes shift the emphasis from form to content, that they recognize

merit in any form in proportion as it aids in clarifying the individual case, and demerit in proportion as it exhibits aimless wandering, irrelevant matter, redundance, disjointing of related facts, or otherwise clouds the issue.

"3. That the technic of history taking be regarded as an intellectual rather than a mechanical process. Histories should exhibit uniform clue-mindedness rather than a uniform system of headings and paragraphing. The record of important phenomena, such as weight loss, blood vomiting or edema, should show in the immediate context the effort to assemble the available historical evidence for or against the various possibilities suggested.

"It will be argued that such freedom in composition may be all right for mature clinicians but not for students; that the latter will overlook important data if they are not required to include all the conventional paragraphs. Of course they will; but so are they under the present burdensome regime; and it is better that they miss some of the kernels of grain while actually looking for them than that they miss them through being more occupied by an elaborate sorting of the chaff."

It is probable that more than one hospital pathologist has been impressed with the multitude of careful and varied preoperative studies carried out during the preparation of the fifty cases required for obtaining the coveted F.A.C.S. and likewise somewhat nonplussed to note their abrupt cessation after the initiation ceremonies are a matter of history.

What conclusion is a Resident Physician to form from such a happening: that such studies are valuable and necessary; that they were based upon real indications in fifty consecutive cases which indications were then no longer to be found; or that they are simply "the bunk"?

What value can he attribute to the histories the sole reason for writing which is that the patient is to be discharged, or the "progress notes" compiled when he has gone home?

It is undoubtedly true that Resident Physicians have been to a greater or lesser extent benefited by the compulsory reformation thrust upon hospitals. They have cause to be thankful that the days have passed when all the emphasis was laid upon the duty the interne owed to the hospital and precious little upon what the hospital owed to the interne.

But the millennium is not yet here and one wonders what would accrue if it became the custom for internes to report, when their hospital days were over, to their Dean who advised them in their student days as to interneship, their honest opinion as to their experience, their summation as to what they learned and from how great a proportion of the staff, and to what exact degree, as testified to by actual experience, the "approved" hospital justifies its rating.

It is obvious that by far the greater number of those who embark upon the interne year receive their training, not in great medical centers, not in well-equipped and well-manned clinics, not in hospitals with teaching affiliations, but in the average hospital of moderate size. Nor is it always true that those under whom they serve, with whom they are associated in this formative period which often does and always should exert a profound and powerful influence upon the manner in which these young men will later

practice their profession, are necessarily possessed of the teaching instinct or the ability to pass on to others the lessons they themselves have learned in the School of Experience.

If, as a result of his experience, the Resident Physician does not emerge from his hospital year richer in knowledge and enthusiasm, inculcated with the idea of honest, earnest care and thoroughness in the study of his patients, inspired to give to the life work he has chosen the very best that is in him, and thoroughly imbued with the necessity for continual effort to improve his knowledge and ability; and if the lack of these ideals and qualities can be laid at the door of the hospital and its staff, then that hospital has but ill fulfilled its duty toward the interne, has but ill discharged the responsibility which it has assumed toward him, and ill deserved the "approved" status which it doubtless possesses.

And, by the same token, the Dean or the School by whose advice or with whose tacit assent the student seeks and accepts such an appointment, have ill deserved the confidence and belief he has given them.

"Approved" hospitals do not by mere virtue of that fact necessarily become good hospitals nor, and the two are by no means synonymous, efficient centers of postgraduate instruction.

One's travels need not be wide nor his experience with hospitals extensive to grant the truth of this statement.

It is impossible to reach perfection in a single stride; the standardization program, without doubt, was not regarded by its originators as achieving perfection. But there seems reason to wonder if its initial impetus may not have subsided into a gentle stroll along well-defined but narrow pathways; if the early desire for improvements in order to secure approval may not have simmered down to the gentle "complacency of Class-A-dom"; and if its main result is not in some danger of becoming simply a "standard of mediocrity."

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—*R. A. K.*